B1U4 OUTLINE

**Nucleotide Metabolism 1 & 2** (p4-49)

I. Structure and Nomenclature

A. Nomenclature

1. Nucleoside vs Nucleotide (p8)

2. Major Bonds (acid anhydride, phosphate monoester, N-glycosidic)

3. Pyrimidine vs Purine (p9)

a. Substituent numbering

b. Urea units

5. Names (p13)

a. Cytosine vs Cytidine?

B. Structures

1. Bases (Adenine, Guanine, Thymine, Uracil, Cytosine) (p10)

2. Nucleosides (RNA and DNA forms)

a. Sugar ring substituent numbering (p11)

3. Base pairing (H-Bonds) (p12)

C. Others (DNK strx) (p14)

1. Hypoxanthine, Xanthine, Inosine monophosphate (IMP), XMP

E. Abbreviated overview of Nucleotide Synthesis (purines and pyrimidines) (p46)

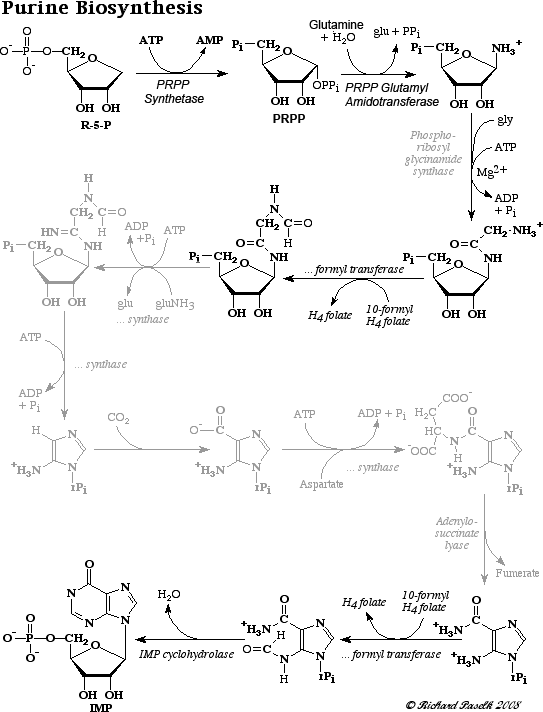
1. Phosphorylation levels *Learning Ob!*

II. Purine Synthesis (full pathway p16)

A. Intro/Overview (p15)

1. Bases normally *de novo*, despite frequency in diet.

2. Two sources of Ribose-5-Phosphate, both from Pentose Phosphate Pathway

 B. Pathway

1. PRPP (phosphoribosyl pyrophosphate) Glutamyl Amidotransferase is 1st committed step for purine synthesis (p16) *Learning Ob!*

2. Glycine adds 3 “atoms” in 1 step. All other steps only add 1 “atom”. (p17)

a. N10-Formyl Tetrahydrofolate (THF) is one of the carbon donors

3. 1st nucleotide formed is IMP. *Learning Ob!*

4. After IMP (Inosine monophosphate) is produced, path splits to form either AMP or GMP (pathway p17)

a. Adenylosuccinate synthetase, IMP dehydrogenase, Adenylosuccinase

5. Origins of purine substituents (p17)

C. Regulation (p18)

1. Steps 1 & 2 inhibited by IMP, AMP, and GMP (p18)

a. Only step 2 is committed step, but step 1 product (PRPP) is mainly used (but not exclusively) by purine synth pathway, so it is regulated too.

2. 1st step from IMP to AMP/GMP branches is inhibited by AMP or GMP (each for its own branch of pathway) (p18)

a. Adenylosuccinate, Xanthosine monophosphate

b. IMP Dehydrogenase, Adenylosuccinate Synthetase

II. Purine Catabolism (pathway p18)

A. Adenosine Deaminase, Xanthine Oxidase *Know these*

B. Final product of pathway: Uric Acid

III. Purine Metabolism Disorders

A. Xanthinuria (p19)

B. Gout (p19-23)

1. Hyperuricemia vs Gout (p19)

2. Causes (p20)

a. Genetic causes

i. PRPP Synthetase

ii. Glucose-6-Phosphatase (Von Gierke’s Disease)

iii. Partial Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRTase) (p21)

3. Treatment

a. Colchicine (p21)

b. Allopurinol (p22)

i. Mechanism (p23)

c. Uricosuric druges (e.g. Probenecid) (p22)

C. Severe Combined Immunodeficiency Syndrome (SCID)

1. Adenosine Deaminase (p23)

2. Purine Nucleotide Phosphorylase (p24)

IV. Purine Antimetabolites

A. Glutamine Analogs (p25)

1. Amidotransferases and CTP Synthetase

2. e.g. Azaserine

B. Purine Nucleotide Analogs (p25)

1. Phosphoribosyl pyrophosphate amidotransferase

2. e.g. Mercaptopurine

C. Antifolates (p26)

1. Dihydrofolate reductase (DHFR)

2. e.g. Methotrexate and Aminopterin

E. Uses of Antimetabolites (p26)

1. Chemo for diff cancers, and some antibiotics.

V. Pyrimidine Biosynthesis

A. Overview (p27)

1. Pyrimidines made from simple abundant compounds; CO2, Aspartate, amide of Glutamine

2. All ring atoms present after 2nd step.

3. Ribose added after the ring is formed (in contrast to purine synth)

4. Source of ribose is PRPP (in both pyrimidine and purine synth)

5. Three major end-products: UTP, CTP, and TMP

B. Main Pathway (diagram p29)

1/2/3. CO2 + Glutamine + ATP → Carbamoyl Phosphate → Carbamoyl Aspartic Acid → Dihydroorotic Acid (DHOA) (p28)

a. Via CAD enzyme complex (does 1st 3 steps) (p30)

i. Carbamoyl Phosphate Synthetase 2 (CPS2)

(a) Structure and “Channeling” (p31)

(b) This 1st step is committed step of pyrimidine synthesis (p35)

ii. Aspartate Transcarbamoylase (ATC) (p30)

iii. Dihydroorotase (DHO)

b. CAA is 1st product where all ring atoms present (p27)

c. Aspartate incorporated into CAA, provides large portion of ring (p32)

4. DHOA → Orotic Acid (OA) (p28)

a. Via Dihydroorotate Dehydrogenase

b. Orotic Acid is 1st pyrimidine, DHOA is not bc doesn’t have correct reduction state

5. OA + PRPP → Orotidine Monophosphate (OMP)

a. Via UMP Synthase (complex that performs step 5 and 6) (p30)

i. Orotate Phosphoribosyl Transferase

ii. Orotidylic Acid Decarboxylase

b. OMP is 1st pyrimidine nucleotide in pathway

6. OMP → UMP + CO2 via UMP Synthase (p30)

a. Uridine Monophosphate is 1st major end-product produced.

b. Branch point for production of either CTP or TMP

C. Branches of Pathway

1. CTP Branch (p32-3)

a. UMP → UDP → UTP → CTP

b. CTP Synthetase, Glutamine

2. TMP Branch

a. UMP → UDP → dUDP → dUMP → dTMP (aka TMP)

b. Ribonucleotide reductase, Thymidylate synthase

c. N5,N10-Methylene Tetrahydrofolate (THF)

D. Regulation of Pyrimidine Synthesis

1. UTP down-regs CPS2 (p34)

2. Carbamoyl phosphate = intermediate in urea cycle too. Must be independently regulated by…

a. Compartmentation (p35)

b. Metabolic Channeling

3. Inhibition by Antimetabolites

a. Glutamine analogs inhibit UTP → CTP via CTP Synthetase (p35)

b. 5-Fluorouracil (5-FU) inhibits Thymidylate synthase (p36)

i. 5-FU converted into 5-fluoro-2’-deoxyuridine-5’-phosphate (FdUMP)

(a) FdUMP irreversibly binds Thymidylate Synthase

ii. Used in treatment of solid tumors

iii. Can be fatal if used in pt with DPD Deficiency (p37)

(a) Prolonged half-life

VI. Pyrimidine Catabolism

A. Pathway (diagram p36)

1. Dihydropyrimidine Dehydrogenase (DPD)

2. Major end products: CO2, NH3, β-Alanine, and β-aminoisobutyrate (p37)

a. β-aminoisobutyrate in urine = indication of excessive DNA catabolism

VII. Pyrimidine Metabolism Disorders

A. Hereditary Orotic Aciduria (p38)

1. Orotate phosphoribosyl transferase, Orotidine-5’-P Pyrophosphorylase, Orotidine-5’-P Decarboxylase

2. UMP Synthase

VIII. Synthesis of Deoxyribonucleotides

A. dNTPs made by reduction of ribose on pre-existing ribonucleotides (p39)

1. All 4 ribonucleotides converted by same enzyme, Ribonucleotide Reductase

B. Ribonucleotide Reductase

1. Reaction (p40)

a. Diphosphate level reduction

b. Thioredoxin

2. Structure (p40)

a. Regulatory sites, catalytic site

3. Regulation (p41)

a. Effect (and target site) of dATP, dGTP, dTTP

b. Pyrimidine nucleoside diphosphates

C. ADA Deficiency and SCID (p42)

1. Deoxyinosine

D. Thymidine Blocks DNA Synthesis

1. Thymidine → dTTP, which blocks reduction of pyrimidine nucleoside diphosphates (NDPs) (p42)

a. ↑ pools of dTTP, dGTP, and dATP, but ↓ dCTP (due to chart p41)

2. dTMP synthesis doesn’t involved ribonucleotide reductase (other dNMPs do) (p43)

a. Thymidylate synthase (p43) *Learning Ob!*

b. Dihydrofolate (FH2), N5,N10-Methylene Tetrahydrofolate (THF)

c. Folic Acid (p44)

i. Analogs (Aminopterin, Methotrexate, Amethopterin)

3. Folate Cycle (p45)

a. Dihydrofolate Reductase (DHFR), FdUMP

b. Serine Hydroxymethyltransferase (SHMT)

i. Principle metabolic source of 1-carbon units *Learning Ob!*

c. Effect of methotrexate and aminopterin

IX. Salvage Pathways

A. Phosphoribosyl Transferase

1. Adds sugar-phosphate in one step (mechanism p47)

2. Enzymes (p47)

a. Adenine Phosphoribosyl Transferase (APRT)

b. Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT)

c. Uracil Phosphoribosyl Transferase (UPRT)

d. No enzyme for Cytosine

3. Lesch-Nyhan Syndrome (p47)

B. Nucleoside Phosphorylase followed by Nucleoside Kinase (p48)

0. Only available for salvage of Thymine and Uracil

1. Phosphorylase step

a. Thymidine + dRibose-1-P → Thymidine + Pi

2. Kinase step

a. Thymidine + ATP → dTMP + ADP

**Meiosis and Gametogenesis** (p50-87)

I. Meiosis

A. Overview (diagram p55)

1. Only in germline cells, in testes and ovaries

2. At least one recombination per homologous pair of chromosomes

3. Meiosis I has reductional division (4C → 2C, 2n → n) (p55)

4. Meiosis II has equational division (2C → C, n remains same)

5. Centromeres only divide ONCE (p55)

B. Meiosis I

1. Prophase 1: replicated chromosomes condense inside nuclear envelope, pair with homologues, form tetrads (p56, p60)

o. Long in humans (~3wks in males, ~12-50yrs in females)

a. Lepotene: Chromosomes begin to thicken and become visible, chromatids not distinguished from each other (p56)

b. Zygotene: Homologous chromosomes enter synapsis, begin to form synaptonemal complex.

c. Pachytene: Synapsis complete, crossing over b/n nonsister chromatids

d. Diplotene: Synaptonemal complex dissolves, tetrads visible; crossover points visible as chiasmata; meiotic arrest occurs here in many species

e. Diakinesis: Chromatids thicken and shorten (they have been doing so for the entire time). At end, nuclear envelope breaks down, spindle begins formation.

2. Metaphase 1: Tetrads held together by chiasmata, chromosomes align at metaphase plate

3. Anaphase 1: Homologous chromosomes separate and migrate to opposite poles

4. Telophase 1: Separation into daughter cells

C. Synaptonemal Complexes (p57)

1. Synaptonemal Complex (p58)

a. Central element, transverse elements, lateral elements

2. Synaptonemal Complex Formation During Prophase I (p58)

3. Chiasmata and Crossing Over (p59)

E. Meiosis II (diagram p59)

1. Prophase 2: chromosomes of 2 daughter cells condense again

a. New spindle forms at right angle to spindle from meiosis I. (p59)

2. Metaphase 2: chromosomes migrate to the metaphase plate

3. Anaphase 2: daughter chromatids separate, centromeres divide, forming new chromosomes

4. Telophase 2: Separation into gametes/haploid cells.

F. Comparison of Meiosis and Mitosis (p60)

II. Male Gametogenesis: ~ 64-day cycle (summary diagram p68, summary text p70-1)

A. Male anatomy

1. Structures (p62)

a. Ductus (vas) deferens, Epididymis, Ductuli efferentes, Rete testis, Tunica albuginea, Seminiferous tubules, Seminiferous epithelium, Testicular lobules, Testis (p62)

b. Corpus cavernosum, Corpus spongiosum, Bulbourethral gland

c. Where are spermatozoa produced? Where are they stored? Where do they mature?

2. Supporting Cells

a. Sertoli Cells (p63)

i. Adluminal and basal compartment

ii. Functions (p68)

(a) Blood-testis barrier

(b) Seminiferous epithelium architecture

(c) Secretory: Androgen Binding Protein, Inhibin, Anti-Mullerian Hormone

(d) Release of sperm

(e) Germ cell development (physical/nutritional support)

(f) Phagocytosis of shed cytoplasm

b. Leydig Cells (missing slides, p69)

i. Found outside seminiferous tubules in intertubular compartment (interstitial cells in the tunica vasculosa)

ii. Produce testosterone in response to luteinizing hormone

iii. Hormonal stimulation necessary for normal spermatogenesis (beyond meiosis). Absence → infertility.

B. Clonality of Spermatogenesis

1. Type A vs Type B Spermatogonia (p64)

2. Primary Spermatocytes → Secondary Spermatocyte

a. Intracellular bridges/syncytium (p65)

3. Spermatids, Spermatozoa, Residual bodies

4. Chromosomal content throughout Spermatogenesis (diagram p65)

C. Phases of Spermatid Maturation

0. Spermatid Structure (annulus? Centriole?) (p66-7)

1. Golgi Phase (centrioles?) (p66)

2. Cap Phase (acrosome?)

3. Acrosomal Phase (nucleus?)

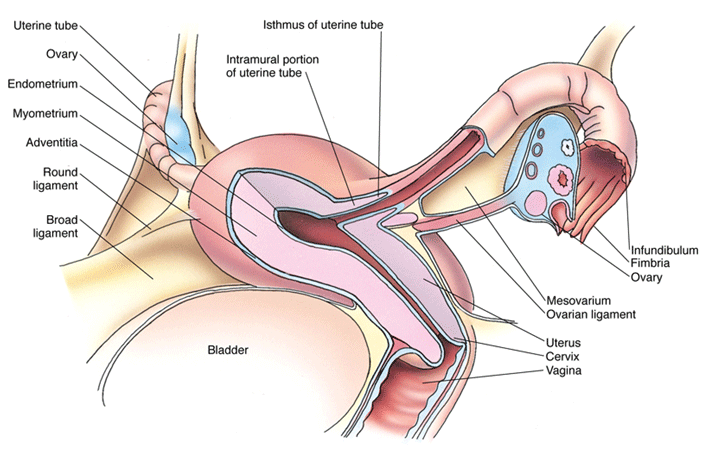
4. Maturation Phase (cytoplasm?)

5. Spermiation (p67)

i. Capacitated

III. Female Gametogenesis

A. Female Anatomy (missing slide)



B. Developmental Timing (missing slide)

1. Oogenesis begins with process of developing oogonia – by transformation of primordial follicles → primary oocytes.

2. ~ 7 million oogonia in 5-month old embryo (maximum number)

3. While still a fetus, development of all primary oocytes arrested in Prophase 1 (diplotene).

4. Females born with 1 million “primordial germ cells”. ~ 300k-400k present at puberty.

5. ~450 oocytes will be released during female productive lifespan, the remainder degenerate and die (atreisia)

a. Atresia (p74)

C. Female Meiosis has 2 Phases (p73)

1. Embryonic Phase

a. Primodrial germ cell migration (extragonadal)

b. Beginning of oogenesis

c. Primary oocytes

d. Arrested development

2. Postnatal Phase

a. Puberty

b. Follicular development

c. 1st meiotic division

d. Ovulation

D. Oogenesis vs Spermatogenesis (p73, p79)

E. Follicular Development (summary diagram p74-5)

1. Primordial follicle, Primary follicle, Multilaminar primary follicle, Secondary follicle, Graafian follicle (p74)

a. Corona radiata (also p76), Corpus luteum, Corpus albicans

b. Antrum, Zona pellucida, Theca interna, Theca externa (p76)

c. Antral Follicles (p76)

2. The Egg (p77)

a. Luteinizing Hormone

b. Secondary oocyte, 1st polar body

c. Cortical granules, perivitelline space

F. Oogenesis (summary diagram p78)

IV. Fertilization/Completion of Female Meiosis (diagram p81, 82)

A. Spermatozoon → capacitated, → oocyte in fallopian tube (upper part) (p79)

B. Spermatozoon attaches to secondary oocyte

1. ZP3 receptor (location?) (p80)

2. Acrosome reaction

a. Acrosin, perivitelline space

C. Spermatozoon passes through zona pellucida, fuses with secondary oocyte. Then… (p79)

1. 2nd meiotic division complete, 2nd polar body extruded

2. Penetration of other sperm blocked (polyspermy)

a. Cortical Granule Reaction (p80)

i. Fast component vs slow component

ii. Zona reaction

V. Zygote

A. First Cleavage Division (p83, summary diagram p86)

1. Pronuclei (~4-6hrs s/p fusion of gametes)

a. EM of pronucleus (mitochondria, nucleolus?) (p84)

b. Breakdown of pronuclear membrane (p83,85)

2. First mitotic division (how long s/p fertilization?) (p83)

**Chromosome Structure & Analysis** (p88-137)

I. Chromosomes

A. Structure: DNA, Histones, and Nonhistone proteins

1. Histones (p93)

a. Compaction

b. Transcribed in nucleus, translated in cyto, imported back into nucleus

2. Nonhistone proteins (NHPs) (p94)

a. Hundreds of kinds; enzymes, regulators, structural

3. Centromere (p95)

a. Kinetochore

b. Satellite DNA (p95)

B. Structural Classification

1. Arms (p vs q) (p95)

2. Metacentric, Submetacentric, Acrocentric, Telocentric (p96)

a. Satellite, Secondary Constriction

b. Nucleolus Organizer Region

C. Chromosome size is *not* proportional to gene content (p100-1)

II. Chromosome Preparation

A. Karyotype

1. Procedure (p98)

a. 5-10mL heparinized blood

b. Lymphocytes vs erythrocytes (which stays?)

c. Phytohemagglutinin (PHA) (how long? Why?)

d. Colchicine or colcemid (fxn?)

e. Hypotonic soln

f. Mounting, air dried

g. Stained

2. Karyotype Documentation

a. 46, XY (p99)

b. Arranged by size and centromere location

i. Exception: HSA21 and HSA22

ii. Sex chromosomes?

B. Comparative Genomic Hybridization (CGH)

1. Procedure (p108)

a. Extract DNA (control & pts)

b. Label c red/green fluorescent

c. Hybridize to microarray, wash

d. Measure separate fluorescence, then measure combined fluorescence

i. Yellow: equal expression

ii. Red: Pt > control

iii. Green: Control > Pt

e. Analyze with computer

C. Florescence In Situ Hybridization (FISH)

1. Procedure (p109)

a. Hybridize fluorescent probe (for specific gene)

b. Fluoresce. Compare number of hybridized regions to control

2. Can use on chromosome spreads or non-dividing cells

D. Spectral Karyotype Analysis (SKY) (p110)

1. Uses chromosome specific probes, each with different fluorescent chromophore (i.e. color).

2. Easily reveals translocations

III. Chromosome Classification – Groups A, B, C, D, E, F, G and Sex (full diagram p103)

Group A: 1, 2, 3. Large metacentric. (p101)

Group B: 4, 5. Large submetacentric (difficult to distinguish from each other)

Group C: 6 through 12. Medium size submetacentric (p102)

Group D: 13, 14, 15. Medium size acrocentric c satellites and secondary constrictions

Group E: 16, 17, 18. Relatively short metacentric or submetacentric.

Group F: 19, 20. Short, metacentric

Group G: 21, 22. Short acrocentric, c satellites and secondary constrictions

Sex Chromosomes: X, Y. (p103)

1. Can be either placed in groups based on size (X → C, Y → G), OR in their own group.

2. Y chromosome does not have a satellite

IV. Chromosome Banding and Nomenclature

A. By Species (p99)

B. Banding (p104-5)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Band | Technique | Pattern | Composition of DNA | Chromatin Type | Timing | Transcriptional Activity |
| G-Bands (Q-bands, quinicrine) | Giemsa (most common) | Ex p106 | AT-rich | Hetero | Mid – late S | Low, tissue specific genes |
| R-Bands | Various | Reverse of G.  Ex p107 | GC-rich | Eu | Early S | High, housekeepers. |
| T-Bands | Various | Telomeres | - | - | - | - |
| C-Bands | DNA extraction, then Giemsa | Centromeres, distal Y. Ex p107 | AT and some GC | Hetero | Late S | Absent |
| NOR | Silver stain | Nucleolus organizer centers | - | - | - | - |

C. Nomenclature

1. Chromosome #, Chromosome arm, Region #, Band #, (period) Sub-band number (p105)

e.g. 1q12.2 (p106)

2. Numbering of Chromatid bands (p106)

a. #s increase as they → telomeres.

V. Chromosomal Abnormalities

A. Overview

1. Causes: extra, missing, rearrangements of chromosomes (p91)

2. Results: Spontaneous abortions, mental disability, congenital anomalies, infertility, cancer

3. Need for chromosomal analysis; will also need genetic counseling (p92)

a. Problems in early growth/development

b. Multiple stillbirths or neonatal deaths (familial?)

c. Fertility problems

d. Family history

e. Neoplasia

f. Pregnancy in women of “advanced age”

4. Fate of a million implanted embryos (p93)

a. 850k live births

i. 833k children, 17k perinatal deaths

(a) of live children, 5k chromosome abnormalities

(b) Male sex chrom aneuploidy most common

(c) Trisomy 21 most common somatic

b. 150k spontaneous abortions

i. 75k chromosome abnormalities

(a) ~50% triosmics

B. Numerical Abnormalities

1. Ploidy Level vs Somic Level (p110)

o. Heteroploid: Any # of chromosomes other than 46. (genetics book p65)

i. Euploid: Exact multiple of haploid # chromosomes.

ii. Aneuploid: Heteroploid that is not euploid.

a. Triploid, Tetraploid

i. All triploidies are incompatible with life

b. Aneuploid

i. Monosomic, Disomic, Trisomic, Tetrasomic (p111)

2. Triploidy (p112)

a. Most often due to dispermy, but can also due to nondisjunction in meiotic divisions of either gamete

i. Diandric vs Digynic

(a) Hydatidiform mole.

b. Phenotypes (p113)

i. Maternal vs Paternal triploids

ii. Early spontaneous abortion, low birth weight, small trunk to head size, syndactyly, multiple congenital abnormalities

c. Mechanism

i. Maternal nondisjunction (p114)

ii. Dispermy (p114)

3. Tetraploidy (p112)

a. 92, XXXX or 92, XXYY. Suggests failure of an early cleavage division in zygote.

b. Deleterious (p119)

4. Aneuploidy

a. Trisomy Mechanisms

i. Nondisjunction Meiosis 1 (p115)

ii. Nondisjunction Meiosis 2

b. Trisomy and Monosomy Rescue (p118)

i. Uniparental Disomy

c. Clinical Significance (p120)

i. Lethality of a particular aneuploidy correlated with gene content of chromosome involved.

(a) Gene rich: 1, 3, 6, 11, 12, 17, 19, 22

(b) Gene poor: 8, 9, 13, 18, 21

5. Mixoploidy (p116), Mosaicism (p116-7) and Chimera (p116)

6. Sex Chromosome Aneuploidies (p122)

a. One of most common aneuploidies (1/500 live births)

b. Often less clinically severe, more compatible with life than autosomal disorders.

i. Due to x-inactivation process and/or small # Y-linked genes

7. Examples Compatible with Live Birth

o. Edward Syndrome (Trisomy 18) & Patau Syndrome (Trisomy 13) (p119)

i. Not discussed & no in-depth info, but listed in “key points” section

a. Down Syndrome (Trisomy 21) (p120-1)

b. Trisomy 16 (p121)

c. Klinefelter Syndrome (p122)

d. Turner Syndrome (p123)

C. Structural Abberations

1. Overview

a. All result from chromosomal breakage.

i. Improper repair/rejoining can happen

b. Rate of breakage increased by…

i. Ionizing radiation (e.g. xrays), rare inherited conditions, chemical mutagens

2. Types (diagram p125)

a. Deletions

i. Terminal vs Interstitial (p130)

b. Ring Chromosomes

c. Inversions (p130)

d. Duplications (intrachromosomal) (p130)

e. Acentric Fragments

f. Dicentric Fragements

g. Translocation

i. Reciprocal translocation (p126)

(a) Balanced reciprocal translocations

ii. Consequences of reciprocal translocation (p127)

(a) Quadravalent structure

iii. ABL1 and BCR translocation (p127)

(a) Philadelphia Chromosome

(b) FISH assay (p128)

iv. Robertsonian Translocation (p129-30)

3. Contiguous Gene Syndromes (Segmental Aneusomy Syndromes)

a. Common features (p131)

i. Small chromosomal segments, generally fxnally unrelated but close proximity

ii. Variable phenotype due to variable breakpoints

iii. Occurrence usually sporadic (non-inherited), but may run in families

iv. Caused by unequal crossing-over between misaligned sister chromatids or homologous chromosomes containing copies of a repeated sequence

b. Williams Syndrome (p132)

c. Cri-du-chat (p134)

**Nucleic Acids** (p138-204)

I. Chromatin Structure

A. Overview

1. Packing ratio 104 (p144)

2. Chromatin: Nucleoprotein complex in nucleus

B. Packing

1. DNA → Beads-on-string → 30nm Fiber → Loop → Rosette → Coil → Chromatid (p145)

C. Nucleosome: Octamer of core histones complexed c ~146bp DNA

1. Histones

a. (+) charge (basic) (which AAs?) (p147)

b. H2A, H2B, H3, H4: 2 of each per octamer

i. H3 and H4 have amino-terminal tail that sticks out (so what?) (p148)

c. H1: one per octamer in the linker region (p147)

2. 1.6 loops of DNA around octamer (p147)

D. Functions (p149)

1. Highly dynamic strx, changes during division and response to DNA damage

2. Chromatin Remodeling: Inactive genes tend to be more condensed, limits accessibility. (p150)

3. Histones → acetylation, phosphorylation, methylation, ubiquitination

II. Structural and Physical Properties of DNA

A. Components of Nucleic Acids

1. Ribose vs Deoxyribose (numbering?) (p150)

2. Phosphate backbone: 3’-5’ phosphodiester linkages (structure?) (p151)

3. Bases (structures? Numbering?) (p151)

4. β-Glycosidic linkage has lots of rotation (p152)

B. Structure

1. General Characteristics

a. Polarity (which way?) (p152)

b. Watson-Crick base pairing (H-bonds?) (p153)

c. Chargaff’s rule (p153)

d. Complementary (p156)

e. Bases are relatively hydrophobic, perpendicularly stacked from backbone (p159)

f. 3.4 Å between bps, 34 Å per complete turn, 20 Å helix width (p159) *Know!*

i. Helix stabilized by H-bonds and base stacking interactions

2. Different Forms (p157)

a. A Form: Compact, wider R-hand helix

b. B Form: Normal DNA, most stable strx under physiological conditions

i. Minor groove vs Major groove (p160)

(a) Important in drug binding (why?)

c. Z Form: L-handed, elongated helix (p157)

3. In Nature, DNA may be… (p161)

a. ds linear

b. ds circular (e.g. mitochondrial, bacterial)

i. Relaxed, supercoiled, catenated

c. ss linear (e.g. some viral)

d. ss circular (e.g. some viral)

4. Supercoils: can be induced by replication (p162)

C. Properties of DNA and Lab Tests

1. Buoyant Density (p163)

a. Higher GC content, higher density.

b. ssDNA forms less extended structure, packs better, more dense.

c. Centrifugation and relative densities (protein, DNA, RNA?) (p164)

2. UV Absorption and Hyperchromic Shift

a. Bases absorb at 260nm (p165)

b. Absorption in ssDNA > dsDNA (p166)

i. How is this useful? (p167)

3. Melting Tempurature (Tm)

a. Temp at which ½ of DNA is ssDNA (p167)

b. Higher GC → Higher melting temp (due to favorable/stable base stacking)

c. Ionic strength dependent (mono/divalent cations stabilize dsDNA)

d. Sequence (i.e. base stacking) influence stability

III. Agents that Interact with DNA

A. Intercalating Agents: typically hydrophobic planar ring strx (p170)

1. Ethidium Bromide (DNA visualization stain)

2. Doxorubicin and Daunorubicin (p171)

a. Anthracyclene ring

b. Bisdaunomycin (p172)

i. Binds DNA c 100x affinity than “parent” compound

3. Actinomycin D (p173)

a. Preferentially binds GpC sequences, intercalates b/n G and C

B. Minor Groove Binding Agents (p174)

1. Netropsin (antimicrobial) (p175)

a. Prefers AT rich regions

2. Mitomycin C (antineoplastic drug) (p174)

a. Prefers CG sites. Can form crosslinks

3. Hoechst 33258 (DNA dye)

C. Crosslinking Agents (p176)

1. Platinum based drugs (anticancer, bind specific sequences in DNA)

2. Can form…

a. Intrastrand adducts (GG, AG, or GXG sites)

b. Interstrand crosslinks (GC sites)

i. Covalent link at N7 position

3. Cisplatin (p177)

D. DNA Binding Proteins

1. Can recognize major and minor groove; pattern of bases and H-bonding sites (p177)

a. Transcription factors

b. DNA Replication Initiators

i. Origin recognition proteins, ssDNA binding proteins, helicases

c. DNA Repair Initiators

i. Glycosylases, Damage Recognition Factors

2. Trp Repressor (p178)

3. Amino Acids (p179)

a. AT site, Major groove: Glutamine or Asparagine

b. GC site, Major groove: Arginine

4. TATA Binding Protein (TBP) (p180)

5. Cro Protein (λ Phage) (p181)

a. Interacts with both major and minor groove as a dimer

E. Helicases

1. Other Unusual DNA Structures (p182)

a. Triplex DNA, Tetraplex DNA, Parallel strand DNA, Hairpin, 3 and 4 way junctions

b. Hoogsteen base pairings (p182)

2. Tetraplex (G4) DNA (p183)

a. Needs runs of at least 3 Gs

b. Frequent in telomeric sequences (TTAGGG)

c. Can be formed during replication or recombination, blocks progress (p184)

3. Diseases

a. RecQ Helicase Family (p184)

b. Bloom’s Syndrome (BLM Helicase) (p185)

c. Werner’s Syndrome (WRN Helicase) (p186)

IV. Restriction Endonucleases and RFLP

A. Classes of Eukaryotic DNA (p187)

1. Single copy, Middle-repetitive, Repetitive (level of duplication? Gene-products?)

2. Gene Families (p187)

a. Duplicated genes

b. Pseudogenes

3. SINEs (Short Interspersed Repetitive Elements) (p188)

a. Alu sequences (what % of genome?)

4. LINEs (Long Interspersed Repetitive Elements) (p188)

a. Reverse transcriptase and RNAase H

b. Genome expansion (what % of genome?)

5. Satellite sequences (p188)

a. Can be studied with buoyant density techniques

6. Junk DNA

B. DNA Repeat Expansion Disorders

1. There are many examples (p189)

2. Fragile X Syndrome (p190-2)

3. Huntington’s Disease (p193)

C. Restriction Endonucleases

1. Recognition sequence is often (but not always) a palindrome (p193)

a. Blunt vs Staggered end

2. Bacterial defense mechanism against viruses.

a. Methylated DNA

3. EcoR1, Pst1, Hae3 (p194)

4. Restriction Map (p194-5)

D. Restriction Fragment Length Polymorphisms (RFLP)

1. Measured b/n two alleles or two individuals (p195)

2. Differences in BPs b/n individuals occurs at 1/1000bp

3. Detection of Sickle Cell Hb Gene (p198-9)

V. Southern and Northern Hybridizations

A. Southern Hybridization/Blot (p196)

1. Alkaline conditions, nitrocellulose filter, radioactive probe (migration direction vs charge?)

2. Utility of technique? (p197)

B. Northern Hybridizaiton/Blot (p197)

C. Other blots (p198)

e.g. Western

VI. Structure and Physical Properties of RNA

A. Major Components of RNA (p199)

1. Bases: Cytosine, Uracil, Guanine, Adenine (strxs?)

2. Ribose

a. “Confers lability in very alkaline solutions” (p200)

B. Structure

1. Complement of template DNA strand (p200)

2. Many types of structure exist (ss, duplex, 5S, RNA-DNA hybrids)

a. ssRNA will form 2° structure with lowest energy content (p201)

i. Thus, ssRNA c complementary portions typically → dsRNA

b. Some have non-canonical base pairing (p202)

i. e.g. Hoogsteen, Reverse-Hoogsteen, G-U pairs in tRNA, base triples.

c. Folding of large RNAs = complex (p202)

i. Complex H-bonding patterns result from 2’ OH on ribose.

ii. RNA more flexible than DNA, can form strx not seen in DNA

**DNA Replication I & II** (p204-256)

I. Dideoxy DNA Sequencing

A. Dideoxy Nucleoside Triphosphate (strx?) (p205)

1. Normal dNTP incorporation and effect of ddNTP

2. Modified nucleosides as Drugs

a. Acycloguanosine (Acyclovir) (p206)

i. Disease? Mechanism?

b. Cytosine Arabinoside (AraC, Cytarabine) (p206)

i. Disease? Mechanism?

c. Azidothymidine (AZT) (p206)

i. Disease? Mechanism?

B. Sequencing (p207-209)

1. ddNTP concentrations?

2. Gel results and interpretation (p208)

3. Current methods (p210)

II. Polymerase Chain Reaction (PCR)

A. Requirements for PCR (p210)

e.g. Taq Polymerase, Thermocycler

B. Uses of PCR (p211)

e.g. Pathogen ID, Prenatal diagnosis, Forensic science, Recombinant DNA tech

1. PCR Fingerprinting (p213)

a. Satellite sequences

b. Variable Number of Tandem Repeats (VNTR)

C. PCR Procedure (p211)

III. Meselson Stahl Experiment

A. Models of DNA Replication

1. Conservative, Semiconservative, Dispersive (p214)

B. Experimental Procedure (p215)

1. 15NH4Cl vs 14NH4Cl, Heavy vs Light

2. CsCl (Dense salt), Ultracentrifuge

3. Results from 1st generation? From 2nd generation? (p217)

a. Expected results from conservative and dispersive?

C. DNA Replication is semiconservative.

IV. Mechanism of DNA Synthesis

A. 5’ → 3’

1. Driven thermodynamically by hydrolysis of pyrophosphate (p218)

2. Nucleophilic attack by 3’ OH on α-phosphate of entering dNTP, PPi is leaving group

3. Mg2+ coordinates new dNTP and helps c nucleophilic attack (p219)

a. Asp residues in DNA polymerase

B. Template and primer needed

1. Processive/Processivity (p219)

a. Difference reflected by size of product formed (p220)

i. DNA Polymerase III > DNA Polymerase I

C. Requires dNTPs, Mg2+, 3’-OH, DNA template (p221)

V. Enzymatic Activities in DNA Synthesis (overview p233)

A. Primase (p221)

1. Specialized RNA polymerase that synths RNA primer for DNA replication

B. *E. Coli* DNA Polymerase I & III (p221)

1. 3’→5’ exonuclease activity (proofreading) (both Pols have this fxn) (p223)

a. 1° source of “high fidelity” of DNA replication (1 error per 109 bp)

b. Enzymes lacking this (e.g. HIV Reverse Transcriptase) have high error rates

2. DNA Polymerase I – Lagging strand synth (p221)

a. Moderately processive (~20nts before dissociating)

b. Slow rate of fxn (~10nts/sec)

c. Has 5’→3’ exonuclease activity

i. Removes primer

ii. Fills gaps between lagging strands

d. DNA repair function

3. DNA Polymerase III – Leading strand synth (p221)

a. Highly processive

b. High rate of fxn (~1000nts/sec)

c. Lacks 5’→3’ exonuclease activity

C. DNA Ligase (p222)

D. Topoisomerases

0. Overview (p229)

a. Form tyrosyl DNA-protein covalent intermediates

b. Targets for anticancer drugs and antibiotics

i. Etoposide and Camptothecins (mechanism?)

1. Topoisomerase I

a. Mechanism (ATP requirement? How many supercoils removed?) (p230-1)

2. Topoisomerase II

a. Mechanism (ATP requirement?) (p232)

i. Removes positive supercoils ahead of fork

ii. Introduces negative supercoils in relaxed DNA

iii. N-gate vs C-gate

b. Catalyze catenation-decatenation reactions

VI. Replication Initiation and Replication Forks

A. Initiation of Replication in *E. Coli*

1. oriC, origin-binding proteins, helicase, single-strand binding proteins (SSB) (p224)

2. DNA Polymerase III complex (dimer), primase

3. Bidirectional replication fork movement (p227)

B. Leading vs Lagging Strand (p225)

1. Okazaki fragments (p226)

a. ~200bp in eukaryotes, ~1000bp in prokaryotes

2. Okazaki experiment (p226) *Know, L.O.*

a. Semi-discontinuous model.

b. 3H-Thymidine, short cycles of replication

i. ½ of DNA label found in short chains, other ½ in long molecules

ii. Longer time → more long molecules, fewer short chains

3. Replication induces supercoils (p228)

a. Unwound by topoisomerases (e.g. DNA Gyrase)

C. *E. Coli* Replication Fork (picture p227)

1. β-subunit sliding clamp, SSB, Ligase, Primase, DNA Pol I & III

2. DNA Gyrase (p228)

3. Helicase separates the strands to allow replication

4. Bending/doubling back of lagging strand allows synthesis in both directions by only one enzyme complex.

D. Eukaryotic Replication Fork (picture p235)

1. Replicons (how many in prok?) (p233)

2. Polymerases

a. Polymerase δ and ε (p234)

i. Leading/lagging strand synthesis (controversy over which is which)

ii. Have 3’→5’ exonuclease activity

iii. Equivalent to Polymerase III in prok.

b. Polymerase α/Primase Complex (p234)

i. Primer synthesis (RNA and DNA here, but only RNA in prok.)

ii. No 3’→5’ exonuclease (errors made are removed by δ)

iii. Equivalent to Primase in prok.

c. Polymerase β (p234)

i. Repair (equivalent to Pol I in prok), but no 3’→5’ exonuclease

d. Others (p234)

i. Polymerase γ: mtDNA synth

ii. Polymerase ζ: Replication past thymine dimers

3. Proliferating Cell Nuclear Antigen (PCNA) (p235)

a. Trimeric clamp around DNA, assoc’d c Polymerase δ and ε

b. Increases processivity of Polymerase δ and ε

4. Replication Factor C (RFC) (p235)

a. Helps load/unload Polymerase δ and ε from DNA strand, particularly when they hit a primer.

5. RPA (RP-A) (p235) *an SSB*

6. RNAase H1/Fen1 (p235)

a. Removes RNA primer from lagging strand

b. Assoc’d with DNA Ligase I

7. Summary of Process (for Lagging Strand) (p235)

VII. Telomeres

A. Telomeres Overview

1. Sequence (TTAGGG) (p238)

a. G-G Hoogsteen base-pairing

2. Telomerase (enzyme bound RNA component) (p238)

3. ~104 bp in humans, heterogeneous

4. Functions (p239)

a. During replication?

b. “Aging” → Senescence

c. Protects chromosome ends from damage repair systems

B. Telomerase in disease (p239)

1. Activity in germline cells and high-turnover cells

a. In 80-90% of tumors too

2. Hayflick limit

C. Telomere Loss and Telomerase Mechanism (p240-1)

D. Unusual Telomeric Structures (purpose?)

1. Tetraplex (p242)

2. T-Loop (Telomere Loop) and D-Loop (p243)

a. TRF2, TRF1

VIII. DNA Repair Mechanisms

A. Photoenzymatic Repair

1. Pyrimidine dimer (produced by UV light) (p245)

a. Thymine-thymine cyclobutane dimer, 6-4 pyrimidine photoproducts

2. Restored by Photolyase

a. Not present in humans, only lower organisms (p246)

b. Enzyme is activated by light (p247)

B. Base Excision Repair

1. Mostly for repairing dmg of single base (not large chunks) (p247)

a. Chemical or radiation-induced dmg typical

b. Also keeps uracil and hypoxanthine out of DNA

2. Mechanism (p248)

a. DNA Glycosylases (various for various bases)

b. Endonuclease

c. DNA Polymerase (β in humans, Pol I in E. Coli), DNA Ligase

C. Nucleotide Excision Repair

1. Repair of bulky endogenous DNA damage (e.g. UV dmg, smoke mutagens) (p250)

2. Mechanism (p250)

a. XPA, XPC, RPA recognize dmg site

b. TFIIH (multiprotein complex) with helicases (XPB and XPD)

c. Endonucleases XPG and XPF-ERCC1

d. Polymerase δ and ε, DNA Ligase

3. Prokaryotic vs Eukaryotic NER Pathway (p251)

D. Mismatch Repair

1. Corrects errors in DNA synthesis (e.g. incorrect base pairing, polymerase “stuttering”) (p252)

2. Mechanism

a. MutS: MSH2, MSH3, MSH6 (p253)

b. MutL: MLH1, PMS2

c. Recognition of new strand (methylation in bacteria) (p253)

i. Hemimethylated DNA

d. MutH, Exonuclease, DNA Helicae, DNA Polymerase III (p254)

IX. DNA Repair Diseases

A. Xeroderma Pigmentosum (nucleotide excision repair defect) (p249)

B. Hereditary Nonpolyposis Colon Cancer (HNPCC) (mismatch repair defect) (p252)

C. Ataxia Telangiectasia (ATM protein defect; DNA damage signaling) (p255)

D. Fanconi’s Anemia (chromosome instability defect and DNA repair) (p255-6)

**Folates and B12** (p257-277)

I. Vitamin B12 (Cobalamin)

A. Structure (picture p259)

1. Corrin ring c cobalt (p260)

a. Difference in geometry confers specificity in metal binding (compared to heme)

b. R-group site

2. Dimethylbenzimidazole ribonucleotide

3. Substituents (p260)

a. –CN (cyano): Cyanocobalamin

i. Most stable form, used as commercial vitamin

b. –OH: Hydroxycobalamin

i. Commonly ingested form

c. –CH3: Methylcobalamin

i. Coenzyme for methionine synthase

d. 5’ deoxyadenosyl: Deoxyadenosylcobalamin

i. Coenzyme for methylmalonyl CoA mutase

B. Function: Coenzyme for two enzymes/reactions

1. Methylmalonyl CoA Mutase (p261)

a. Methylmalonyl CoA → Succinyl CoA (for TCA cycle) in mitochondria

i. Metabolic big picture (p263)

b. Uses deoxyadenosylcobalamin

2. Methionine Synthase (p262)

a. Homocysteine → Methionine in cytosol

i. Methycobalamin acts as methyl donor

ii. Methyl-THF

b. Reaction Pathway (p262-3)

i. Methionine Cycle (p264)

ii. S-Adenosylmethionine (SAM, AdoMet)

c. Deficiency

i. Homocystinuria (p262)

ii. Reconversion of methyl-THF to THF (p264)

C. Uptake of B12

1. Digestion

a. R-binder (from where?), Intrinsic Factor, Transcobalamin 2, Transcobalamin 1 (p266)

2. Storage (p267)

a. Liver, bound to transcobalamin 1

i. 3-5yr supply

b. Richest source is meat

c. RDA: 3µg/day

D. Pernicious Anemia (p265)

II. Folates

A. Structure (p268)

1. Major Parts

a. Pterin Ring, p-Aminobenzoic Acid, Glutamate

2. Three Major Variables in Various Forms of Folate (p268)

a. # of glutamates

i. In circulation vs in cells (p269)

(a) γ-carboxyl group

ii. Functions (p269)

b. Reduction state of pterin ring (p269)

i. 3 oxidation states: Folate, dihydrofolate (DHF), tetrahydrofolate (THF)

(a) Active form?

ii. Dihydrofolate reductase (DHFR) (p270)

(a) NADPH

c. Addition of 1-carbon units to ring

i. Where are 1-c groups bound? (p270) *N5, N10, or both*

B. Function

1. Table of Reactions (p271) *Know all of them*

2. Diagram of Reactions (p271)

C. Uptake

1. Digestion (p272)

a. Source? Transport?

2. Requirements (p272)

a. RDA: 200µg/day

b. 5-10mg stored in liver (sufficient for how long s dietary folate?)

c. Pregnant women RDA?

D. Deficiency

1. Major health problems (p273)

a. Homocysteinemia (methionine synthase) (p274)

i. CVD risk.

ii. Folate or B12 deficiency

b. Neural tube defects (nucleotide synthesis) (p274)

i. Folate or B12 deficiency

c. Megaloblastic anemia (nucleotide synthesis) (p275)

i. Megaloblasts (location?), macrocytic anemia

ii. Folate or B12 deficiency

(a) Dx tests to differentiate? (p276)

2. Folate Trap Hypothesis: Why B12 deficiency → effective folate deficiency (p273)

E. Sulfa Drugs (p277)

1. Sulfanilamide, p-Aminobenzoic Acid, Bactrim (sulfamethoxazole and trimethoprim)

2. Mechanism of action?

**RNA Transcription I & II** (p280-316)

I. RNA Transcription Overview

A. RNA is the complement of the template strand of DNA (p281)

1. Sense vs antisense

B. RNA Polymerase

1. Initiation and elongation of RNA chains (p281)

2. 5’→3’

a. Template is read 3’→5’ (p282)

3. Prokaryotic RNA Polymerase (p281)

a. Makes mRNA, tRNA, and rRNA.

b. 4 subunits: α2, β, β’

4. Eukaryotic RNA Polymerase

a. Different pols for each class of RNA (exact classes covered later in lec)

5. RNA Polymerases have fewer correction mechanisms than DNA Polymerases (p283)

a. Why?

C. Mechanism

1. Free 3’-OH nucleophillic attack on α-phosphate of entering NTP (p282)

2. DNA totally conserved

3. Transcription bubble moves down gene as RNA is made

4. No primer required (p283)

D. Sequences

1. Promoters (p284)

a. Eukaryotic vs Prokaryotic promoters (p285)

i. Critical control sequences typically located where (upstream or down?)

ii. Sp1

2. Terminators (p284)

3. Consensus Sequences (p284)

a. Pribnow box

b. TATA box

II. Prokaryotic Gene Expression and RNA Synthesis

A. Initiation: Occurs in response to environmental stimuli (p285)

1. Sigma Factor, Holoenzyme (p286)

a. When does sigma factor leave?

2. Translation/Transcription can occur simultaneously (p292)

B. Termination

0. “Specific termination mechanisms can work independently or in concert” (p287)

1. Hairpin structure (p287)

a. 3’ end of RNA transcript.

b. Mechanism?

i. GC rich sequence, AU rich sequence

2. Rho protein (p288)

a. Hexamer, ATP-dependent

C. Lac Operon

1. Organization (diagram p289)

a. LacI: inhibitor

b. Promoter and Operator sites

i. Polycistronic

c. LacZ: β-galatosidase

d. LacY: a permease

e. LacA: thiogalactoside transacetylase (fxn unknown)

2. Induction of Lac Operon

a. Function of β-galactosidase (graph p289)

b. Products of β-galactosidase (p290)

3. Regulation (p290-1)

a. Repressor proteins, 1,6-Allolactose

b. CAP protein (p291)

D. Prokaryotic Transcription Inhibitors (p292)

1. Rifampicin: binds/inhibits β subunit of prok RNA polymerase.

a. Used for TB

2. alpha-Amanitin: inhibits both euk and prok RNA polymerase II and III (II>III)

a. From mushroom (*Amanita phalloides*)

3. Actinomycin D: inhibits euk and prok transcription by intercalation in DNA, blocks RNA pol.

III. Eukaryotic Gene Expression and RNA Synthesis

A. Eukaryotic RNA Polymerases (p293)

0. Classes of Eukaryotic RNA (p293)

a. rRNA, mRNA, tRNA, snRNA, miRNA

1. RNA Polymerase I

a. 45S rRNA precursor, in the nucleolus

2. RNA Polymerase II

a. mRNA, miRNAs, most snRNAs, in the nucleoplasm

3. RNA Polymerase III

a. tRNA, 5S rRNA, some snRNAs, in the nucleoplasm

4. mtRNA Polymerase

a. all types of RNA in mitochondria

B. Eukaryotic Activation of Gene Structure

0. Review

a. Chromatin structure, histones (p294)

b. 30nm fiber (p295)

1. Histone Regulation

a. Less dense → more expression. Typically uncompressed via acetylation (p295)

b. Histone Acetyltransferases (HATs) and Histone Deacetylases (HDACs) (p295)

c. Acetylation mechanism (p296)

i. Lysine on N-terminal tails

ii. Interaction with negative charge phosphate backbone

d. Histones are also methylated/demethylated at specific positions (p297)

i. Effect can be activating or repressing depending on position

2. Chromatin Activation

a. Coactivators and Corepressors (p297)

i. Some have HAT or HDAC activity (respectively)

b. Topoisomerases found assoc’d with active genes (nothing else said abt them) (p297)

c. DNA Methylation (p298)

i. Hypomethylation → contributes to active transcription

ii. C-5 of cytosine primarily at CG dinucleotides in euk.

iii. Genomic imprinting

3. Epigenetic Regulation (p299)

a. Epigenetic: “A change in gene expression that is heritable but is not due to a change in the DNA sequence.”

b. Usually due to histone modification and DNA methylation

c. Can underlie disease (e.g. CA, inflammatory diseases)

i. Drug target

C. Eukaryotic Initiation of Transcription (Transcription Factors)

1. Overview (p300)

a. Transcription factors: protein factors that activate or repress transcription

i. May bind to specific DNA sequences

ii. Many act as adaptor molecules; mediate signaling b/n other factors and RNA polymerase (coactivators and corepressors)

b. Cis-acting elements vs Trans-acting factors (Wikipedia)

i. Cis-acting element: DNA sequence to which a transcription factor binds. It acts on the same DNA molecule that it is a part of. Cis elements do not code for proteins (e.g. lac operator sequence which is bound by lac repressor)

ii. Trans-acting factor: A molecule or protein that binds to and affects a cis-acting element. Encoded by a trans-acting element DNA sequence from a different gene (but not necessarily diff chromosome).

c. They have 2 domains: DNA binding domain, and transactivation domain (p303)

d. Can activate/repress from long distances from promoter (p304)

i. Adaptor protein

2. RNA Polymerase II-dependent Promoter

a. Can have variable organizations (p301)

i. Inr (initiator region), TATA box, DPE (downstream promoter element)

b. Enhancer elements, proximal elements, gen. transcription machinery (diagram p301)

3. Pre-Initiation Complex

a. TFII (TF2) guides RNA Polymerase II (p302)

i. TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH

ii. Each is complex of proteins

b. TATA Binding Protein (TBP)

i. Component of larger TFIID complex (p302)

ii. *Asymmetrically* binds TATA box, causesunwinding/bending of DNA

iii. Scaffold for other transcription factors to bind.

4. Enhancers and Silencers (p307)

a. Cis-acting elements that are not part of basal promoter

b. Can fxn close to promoter or far, in either orientation, even from within intron

c. Bind transcription factors that are frequently cell-type specific

i. Enhancers → activators, Silencers → repressors

5. Examples of Transcription Factors

a. Steroid Hormone Receptors are transcription factors (p304)

i. Activated how? Role of enhancers? (p305)

ii. Estrogen Receptor and Breast Cancer (p304)

iii. Tamoxifen

(a) Mechanism (p307)

b. Activation of Type 1 Nuclear Receptors (e.g. steroid receptors) (p306)

i. Ligand binding domain (LBD), DNA binding domain (DBD)

ii. Heat Shock Proteins (HSP)

c. Homeotic Genes

i. Homeobox proteins

(a) Conserved, very basic sequence (can bind DNA)

ii. Sequence of genes (for homeobox proteins) corresponds to order of action along anterior-posterior axis of embryo

(a) Individual homeotic genes conserved from flies to humans

(b) Arrangement on chromosomes also conserved

iii. Waardenburg Syndrome (p309)

d. *fosB* Transcription Factor (p310)

i. Dimerizes c Jun family members.

ii. Activated during variety of neuronal responses

iii. Nurturing behavior (knockout mice experiment)

D. Eukaryotic Processing of Transcript

1. Capping of mRNA (p312)

a. Cap-binding protein complexed to translation initiation complex

i. Prok RNAs aren’t capped

b. Fxn?

i. Stabilize/cap mRNA, maybe splicing, maybe export

c. Mechanism (p312)

i. 7’-methylguanylate

ii. Which end is capped? *The 5’ end*

2. Polyadenylation of mRNA (p313)

a. Mechanism

i. Cleavage signal, endonuclease

ii. Which end is adenylated? What happens to RNA Pol?

b. PolyA Polymerase (aka Polyadenylate Polymerase)

3. Introns and Exons (p313-4)

a. Processing Mechanism

b. Exons may contain noncoding regions

c. Not all genes are “split”

e.g. histone genes, some interferon genes, some viral genes

d. Thalassemia Syndromes (p315-6)

i. Sickle cell is not one (why?)

ii. Can result from multiple sources of mutation

e.g. Frameshift mutations, transcriptional control mutations

iii. Can be due to point mutation in splicing junctions

(a) Cryptic sites

**Protein Translation and Post-Translational Modification** (p317-341)

I. Genetic Code and Mutations

A. Genetic Code

1. Start (Met) and Stop codons (p318)

2. Open reading frame (ORF)

3. Degenerate code

B. Mutations

1. Substitution, Deletion, Null, Nonsense, Point, Frameshift (p319)

a. Frameshift cannot be multiple of 3bps

II. Ribosome Composition

A. Ribosome Overview

1. Assemble proteins on their surface (p320)

a. Polysomes

i. Can be free in cytosol or attached to ER membranes

2. Composed of 2 dissimilar ribonucleo-protein subunits

a. Each rRNA species is only found once per subunit

3. All ribosomes in given cell compartment are identical (p321)

a. They are drawn from free pool and used again after their release from mRNA

B. Composition

0. Sedimentation value (higher = larger size) (p321)

1. Eukaryotes (p321)

a. Monomer (80S) = Small subunit (40S) + Large subunit (60S)

2. Prokaryotes (p321)

a. Monomer (70S) = Small subunit (30S) + Large subunit (50S)

3. Each subunit composed of rRNA and proteins (p322)

C. Processing (p322)

1. Ribosomal proteins, rRNA precursor, RNAse III

D. Active Sites (picture p323)

1. 60S: Peptidyl transferase, GTPase, exit site

2. 40S: tRNA site

III. tRNA Structure

A. Structure (picture p323)

1. TψC Loop, Dihydrouracil loop, anticodon loop, CCA terminus (at which end?)

a. “TψC” from Ribothymine – Pseudouracil – Cytosine sequence in loop (genetics p891)

b. Where do AAs attach? (p324)

2. Sequence has numerous modified bases (p323)

e.g. 2’-O-methylcytidine, dihydrouridine, inosine, N6-methylguanosine, etc

IV. Activation of Amino Acids

A. Aminoacyl tRNA Synthetase (p324)

1. *At least* one aminoacyl tRNA synthetase per amino acid

B. Activation and Charging of Amino Acids (p325)

0. 2-step process powered by ATP hydrolysis, produces “charged tRNA”

1. AA + ATP → AA-ribose-P (aminoacyl adenylate) + PPi

2. AA-ribose-P + tRNA → AA-tRNA + AMP

V. Translation

A. Initiation of Prokaryotic Translation

1. 5’ end of mRNA → 30S ribosomal subunit at ribosome binding site (p325)

a. Shine-Dalgarno sequence (mRNA) binds sequence at 3’ end of rRNA (16S) in subunit

b. How long and where (on gene) is SD seq? What abt polycistronic genes?

2. Formyl-Methionine (fMet) (p326)

B. Initiation of Eukaryotic Translation

1. 40S Preinitiation Complex slides on mRNA until Kozak Consensus (describe?) (p326)

a. Sub-optimal sequences and start codons skipped

b. Preinitiation complex includes Initiation tRNA (p327)

i. Met

2. mRNA is activated via removal of 5’ cap and 2° strx (e.g.?) by initiation factors (p327)

3. Codon-anticodon base pairing (polarity of strands?) (p328)

4. 60S Ribosomal Subunit assembles to mRNA and 40S, → 80S complex (p328)

a. A-site (aminoacyl), P-site (peptidyl), E-site

b. Reading frame established

C. Elongation (Eukaryotic)

1. Peptide bond formation (p329)

a. Peptidyl transferase

b. Mechanism

2. Translocation

a. eEF-2 and GTP (p330)

D. Termination

1. eRF and stop codons, peptidyl transferase and H2O (p331)

2. Ribosomal complex dissociation

VI. “Wobble” In Codon-anticodon Recognition

A. Wobble position (p333)

B. Degeneracy of genetic code (p334)

1. Effect on # of tRNAs necessary?

2. Which position is the wobble position (in codon and anti)? Do all codons have wobble?

VII. Inhibitors of Translation (p334)

|  |  |  |
| --- | --- | --- |
| Inhibitor | System | Process Affected |
| Streptomycin | Prok. | Formation of initiation complex |
| Tetracycline | Prok. | Aminoacyl tRNA binding at A-site |
| Erythromycin | Prok. | Binds 50S, inhibits translocation |
| Fusidic Acid | Prok/Euk | Inhibits elongation, binds eEF2/GDP |
| Diphtheria Toxin\* | Euk. | Inactivates eEF2 by polyribosylation |
| Puromycin | Prok/Euk | Aminoactyl tRNA analog, acts as peptidyl acceptor |

A. Diphtheria Toxin (p335)

1. Immunity

2. Mechanism of activation and action

a. EF2, ADP-ribosyl EF2, nicotinamide

VIII. Post-Translational Modification of Proteins

0. Acetylation and Methylation of Histones (from previous lecture)

A. Protein Phosphorylation (p336)

1. Kinases and Phosphatases

2. Serine/Threonine Kinases (most common)

3. Tyrosine Kinases (important in hormone signaling)

B. Membrane Anchoring by Fatty Acid Attachment (Covalent Modification)

1. Ras protein, Palmitoyl CoA (p336)

a. Ras is farnesylated at C-terminus

b. Ras in cancer; as a drug target

2. Src protein, Myristyl CoA (p337)

a. Amide link formation

3. GPI-Anchor (p337)

C. Protein Glycosylation

1. Major biosynthetic fxn of ER

a. Most secreted proteins and cell-surface proteins are glycosylated

2. O-linked Glycosylation (p338)

a. Where is oligosaccharide added? *To -OH of Serine/Threo*

b. Generally has simpler sugars added than N-linked

3. N-linked Glycosylation

a. Glycosyl transferase transfers specific 14-sugar residue from dolichol (p338)

i. Glycosyl transferase = ER membrane bound (p339)

ii. Occurs almost immediately after target residues pass through ER membrane

b. Targets: Asn-X-Thr, or Asn-X-Ser (p338)

D. Proteolytic Processing of Proteins

1. Occurs in Golgi (p339)

2. Cleavage usually next to pairs of basic residues (e.g. Arg-Arg, Lys-Arg, etc)

3. Pre-pro-proteins (p340)

a. Preproinsulin and C-peptide

b. Hydrophobic N-terminus sequence

E. Protein Degradation

1. ATP-Independent (p341)

a. What types of proteins? *Memb-bound & long-lived*

b. Degraded how?

2. ATP-Dependent (p341)

a. What types of proteins? *Abnormal or short-lived*

b. Ubiquitin mechanism

i. ε-amino group of lysines

ii. Ubiquinating enzyme complex

iii. Ubiquitin-dependent protease

iv. Final fate of ubiquitin?

**Inborn Errors of Metabolism** (p407-431)

I. Overview

A. Enzymopathies

1. Almost always recessive (p408)

2. Substrate accumulation or product deficit (majority are accumulation)

a. Diffusable vs macromolecular substrate

3. Can be loss of multiple enzyme activities (uncommon, mostly only 1 enzyme)

a. Due to missing common cofactor (of multiple enzymes), processing enzyme, organelle

4. Similar phenotypes can be produced from deficiencies in different enzymes in the same (or closely related) pathway (p408)

5. Partial defects (e.g. partial HGPRT deficiency + hyperuricemia vs full + Lesch-Nyhan)

B. Onset (p409)

1. Early onset assoc’d c acute crisis in newborn

e.g. Urea cycle defect

2. Late onset assoc’d c chronic metabolic disorder

e.g. Lysosomal storage disease

C. Case Study – Galactosemia (p409-10)

II. Screening

A. Criteria (p411)

1. Treatable

a. Particularly if early treatment crucial to outcome

2. Sufficiently high incidence/prevalence

3. Screening test must have…

a. Low false positive/false negative

b. Low cost

B. Categories of Routinely Screened Disorders. (p408, 411)

1. Amino Acidemias e.g. Phenylketonuria (PKU), Maple-Syrup Urine Disease

2. Endocrine e.g. Congenital Adrenal Hyperplasia (CAH), Congenital Hypothyroidism

3. Fatty Acid Oxidation e.g. Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)

4. Organic Acidemias e.g. Isovaleric Acidemia, Methylmalonic Acidemia

5. Urea Cycle e.g. Argininosuccinic Aciduria (also classified as an amino aciduria)

6. Carbohydrate Metabolism e.g. Galactosemia

7. Lysosomal Storage e.g. Tay-Sachs (not screened in OH, but commonly screened)

8. Others

a. Biotinidase Deficiency

b. Cystic Fibrosis

c. Sickle Cell and other hemoglobinopathies

C. Screening Methods

1. Tandem Mass Spectrometry

a. Good for testing overabundance of specific compounds (p412)

e.g. AA acidemias, Organic acidemias, FA ox disorders

b. Method (diagram p412-3)

i. Separation by mass/charge ratio

ii. Example readout (p413)

2. Other disorders detected with disorder-specific tests (p412)

III. Disorders

A. Acidosis and Alkalosis (p415-7)

1. Acidemia: Blood pH below 7.35. Alkalemia: above 7.45

2. Arterial Blood Gas (ABG) (p417)

a. Respiratory vs Metabolic

i. CO2/HCO3- vs pH

(a) ROME: Resp Opposite, Met Equal

i.e. Low CO2 and low pH → Met.

i.e. Low HCO3- and high pH → Resp

3. Anion Gap (p417)

B. Fatty Acid Oxidation Disorders (p418)

1. Detected by specific acylcarnitines via mass spec

2. Symptoms due to inability to derive energy from FAs

a. Hypoketotic hypoglycemia, fasting intolerance

b. Acute: vomiting, lethargy, coma

c. Abnormal organic acid levels in urine

3. Treatment: high carb diet, avoid fasting

C. Amino Acid Disorders (p418)

1. Wide variety, usually due to defects in catabolism, sometimes due to synthesis

2. Detected by specific metabolites via mass spec (mostly in urine) (list p414)

a. What metabs for PKU, MSUD, homocystinuria?

3. Some overlap in nomenclature b/n organic acidurias and urea cycle disorders (p418)

D. Organic Acidemias (p419)

1. Disorders of AA or FA metabo where nonamino organic acids accumulate in serum and urine.

2. Detected by specific acylcarnitines or organic acids via mass spec (list p414)

3. Symptoms

a. Metabolic acidosis, high anion gap

b. Possibly hyperammonemia (why?)

c. Encephalopathy, vomiting, lethargy shortly after birth

4. Treatment: Restriction of substrates that can’t be metabolized.

E. Urea Cycle Disorders (p419)

1. Main problem is hyperammonemia

a. Also detected by analysis of AAs found in blood/urine

2. Presents in newborns after protein intake

a. Vomiting, lethargy, encephalopathy, brain damage

3. Treatment: Low protein, high-carb diet. Benzoic acid or phenylacetic acid (why?)

F. Glycogen Storage Diseases (p420)

1. Result in excess storage of glycogen

a. Manifest as hypoglycemia and hepatomegaly

2. GSD 1: Glucose-6-Phosphatase Deficiency (aka Von Gierke)

a. Presents c high lactic and uric acid levels

G. Endocrine Disorders (p421)

1. Congenital Adrenal Hyperplasia (CAH)

2. Congenital Hypothyroidism

H. Others

1. Biotinidase Deficiency (p422)

a. Example of a Multiple Carboxylase Deficiency

2. Cystic Fibrosis (p423)

a. Immunoreactive trypsinogen (IRT)

I. Lysosomal Storage Disorders

1. Three major types (p423)

a. Mucopolysaccharidoses (substrate?) e.g. Hurler syndrome, Hunter syndrome

b. Oligosaccharidoses (substrate?) e.g. Mannosidosis, Sialidosis, Fucosidosis

c. Sphingolipidoses (substrate?) e.g. Tay-Sachs, Gauchers, Niemann-Pick Type A

2. Usually normal at birth, symptoms worsen throughout childhood due to accumulation (p424)

a. Developmental regression, hepatosplenomegaly, neurodegeneration.

b. CHERRY RED SPOT IN RETINA for some of the disorders

3. Treatment: Severe forms usually lethal in childhood, but enzyme replacement and bone marrow transplantation may be effective for some.

IV. Working Backwards from Lab Findings to Diseases

|  |  |
| --- | --- |
| Lab Finding | Possible Disease |
| Metabolic Acidosis with Increased Anion Gap | Organic Acidemias |
| Respiratory Alkalosis | Urea Cycle Disorders |
| Hyperammonemia | Urea Cycle Disorders  Organic Acidemias |
| Acylcarnitine Profile Abnormalities | FA Oxidation Disorders  Organic Acidemias |
| Hypoglycemia | Glycogen Storage Disorders  Organic Acidemias  MSUD  FA Oxidation Disorders |
| Quantitative Amino Acid Profiles | “Specific defects in AA metabolism have specific patterns” |
| Urine Organic Acids | “Specific defects in AA metabolism have specific patterns” |

V. Treatments

A. Table of Dietary Restrictions (p426)

1. What restrictions for: PKU, Tyrosinemia, MSUD, Homocystinuria, Galactosemia, UCDs?

B. Cofactors as Treatments (p426)

1. Can be used if residual enzyme activity

2. PKU, Homocystinuria, Methylmalonic acidemia?

C. Enzyme Replacement Therapy (ERT) (p427)

1. Gaucher’s Disease, SCID

2. Mechanism: Terminal mannose residues and macrophages

D. Hematopoietic Stem Cell Transplantation (HSCT) (p427)

1. Microglial cells

2. Comparison c ERT

3. Allogenic source (p428)

E. Triggers (p428)

1. Ingestion of sugars?

2. Ingestion of proteins?

3. Introduction of complementary foods in infants?

4. Infection, fever, fasting, or catabolism?

VI. Case Studies (p429-431)

**Mendelian Genetics** (p347-406)

I. Basics of Mendelian Genetics

A. Terminology

1. Gene (p347)

a. Replicated, transcribed, translated into polypeptide (for mRNA) (p348)

2. Phenotype (p351)

a. “Represents summation of actions/interactions of gene (or genes) c the environment”

3. Allele (p349)

a. Single locus

4. Genotype

o. Can be regarding single locus or to many/all loci (p350)

a. Heterozygous (p352)

b. Homozygous

5. Dominance (p352)

o. “Relationship b/n diff alleles at particular locus c respect to effects on phenotype”

a. Dominant

b. Recessive

B. Crosses

1. F1 (First Filial Generation) (p354)

2. Backcross or Test Cross (F1 x P) (p354)

a. Useful to look at segregation of alleles in one parent w/o the other parent messing up the ratios.

3. Albinism (p355)

a. Tyrosinase

4. Intercross (F1 x F1 → F2) (p356)

C. Mendel’s Laws

1. Segregation (p357)

2. Independent Assortment (p361)

a. Black/Orange Snakes (Two Genes, Different Chromosomes) (p358-61)

i. Phenotypic ratio for intercross of double heterozygotes (p361)

b. 3n genotypic classes per “n” pairs of chromosomes if 1 locus per chrom (p362)

i. 94 billion combinations possible in humans (23 pairs of chromosomes)

II. Pedigree Analysis

A. Symbol Usage (full example pedigree p363)

1. Each row = one generation. Counted by roman numerals

a. Progeny in order of birth, 1st (#1) on left.

2. Square = male, circle = female, diamond = unspecified/unknown

a. Filled symbol = affected phenotype. Unfilled = unaffected phenotype

b. Diagonal slash through symbol = dead

3. Horizontal lines = matings. Vertical lines = progeny

a. Break in horizontal line (b/n mates) = ended relationship

b. Dbl horizontal line (b/n mates) = consanguineous relationship

c. Split vertical line (i.e. upside down Y) = dizygotic twins

d. Split vertical line with crossbar (like coat hanger) = monozygotic twins

e. Coat hanger w/o square or circle underneath = spontaneous abortion

4. Consultand and Proband (p366)

B. Chart for Characteristics of Patterns of Inheritance (p366)

(placed at end of lecture below)

III. Autosomal Traits

A. Autosomal Dominant

1. Pedigree Example (p367)

2. Punnett Square (p368)

a. Progeny of affected parent has 50% chance of being affected

3. Criteria for Autosomal Dominant Trait (p367)

a. Vertical Inheritance

b. No sex bias in progeny (exception: sex-limited disorders; later lecture)

c. No sex bias in transmission

d. Unaffected individuals don’t transmit to progeny (exception: incomplete penetrance; later lecture)

e. Homozygous dominant sometimes much more severe (or lethal) (p368)

i. For other disorders, little difference b/n hetero or homozyg dominant phenotype

4. Characteristics of Autosomal Dominant Traits

a. New mutations occur somewhat regularly (p369)

i. More severe the phenotype, more likely it is a new mutation

ii. Advanced paternal age correlation

b. Frequently structural proteins (e.g. collagens and fibrillin)

c. Complications of patterns of inheritance (p369)

i. Incomplete penetrance, variable expressivity, and gonadal mosaicism

d. Haploinsufficiency (p370)

i. Responsible for some autosomal dominant disorders

e.g. William’s Syndrome, Dyskeratosis Congenita (need to know?)

e. Dominant Negative (p370)

e.g. Marfan Syndrome, Osteogenesis Imperfecta

5. Examples for Humans

a. Marfan Syndrome (p371)

i. Variable Expression

b. Chart of Human Autosomal Dominant Disorders (p372)

B. Autosomal Recessive Inheritance

1. Pedigree Example (p373)

2. Punnett Square (p374-5)

3. Criteria for Autosomal Recessive Trait (p373)

a. Usually affects 1 generation, in a single sibship

b. Parents/offspring of proband usually not affected (usually hetero)

c. Horizontal inheritance

d. No sex bias in progeny

e. No sex bias in transmission

4. Characteristics of Autosomal Recessive Traits

a. Rarer the trait, the more likely parents of child are consanguineous (p376)

b. Frequently affects proteins with enzymatic function

i. Typically no haploinsufficiency

c. Most biochemical disorders are autosomal recessive

5. Examples for Humans

a. Tay-Sachs Disease (p377-8)

i. Tay-Sachs pedigree (p378)

III. Sex-Linked Traits

A. Sex Chromosomes

1. X has more genes than Y (p380)

2. Males are hemizygous for *most* genes on X

B. X-Inactivation (p380)

1. Dosage Compensation

2. Barr Bodies

3. Lyon Hypothesis (p381)

4. Examples

a. Tortoise Shell Cat (p381)

b. Anhidrotic Ectodermal Dysplasia (p382)

IV. X-Linked Traits

A. X-Linked Recessive Inheritance

1. Pedigree Example (p382)

2. Punnett Square (p383)

a. Progeny of affected male (i.e. sons and daughters)?

b. Progeny of carrier female? (p384)

3. Criteria for X-Linked Recessive Trait

a. Incidence much higher in males; sex bias in affected progeny (p382)

i. Males almost always more severely affected

b. No male-to-male transmission (essentially); sex bias in transmission

c. Vertical transmission (can be masked by genders of progeny though)

4. Characteristics of X-Linked Recessive Traits

a. Manifesting carriers (p386)

b. Skewed X-inactivation

c. New mutations occur more frequently in sperm, leading to new carrier females

i. ~1/3 of lethal x-linked disorders due to new mutations (why?)

5. Example for Humans

a. Hemophilia A (p387)

b. Color Blindness (p384)

B. X-Linked Dominant Inheritance

1. Pedigree Example (p388)

2. Punnett Square (p389)

a. Carrier Female

b. Affected Male

3. Criteria for X-Linked Dominant Trait

a. Pattern similar to x-linked recessive, but both male and females affected (p388)

i. Expect 2x affected females as males; sex bias in progeny

ii. Generally, males more severely affected

b. No male-to-male transmission; sex bias in transmission

4. Characteristics of X-Linked Dominant Traits

a. Very rare (p390)

b. Some lethal in utero to males (can mess up expected pattern) (p390)

i. If so, only females would appear affected (no affected males reach term)

ii. Affected females would have fewer sons than daughters and more miscarriages

5. Example for Humans

a. Hypophosphatemia (p390)

\* = allele only, carrier

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Sex Bias in Affected Progeny | Sex Bias in Trans-mission | M to M transm | M to F transm | F to M transm | F to F transm | Transm mode: Horz or Vert |
| Autosomal Recessive | N | N | Y\* | Y\* | Y\* | Y\* | H |
| Autosomal Dominant | N | N | Y | Y | Y | Y | V |
| X-Linked Recessive | Y | Y | N | Y\* | Y | Y\* | V |
| X-Linked Dominant | Y | Y | N | Y | Y | Y | V |
| Y-Linked | Y | Y | Y | N | N | N | V |
| Maternal | N | Y | N | N | Y | Y | V |

**Complicating Factors and Multifactorial Inheritance** (p432-500)

I. Additional Patterns of Inheritance

A. Y-linked Inheritance (Hollandric) (summary table in outline above)

1. Pedigree Example

2. Criteria (p436)

a. Only affects males; sex bias in progeny

b. Only M-to-M transmission

3. Regions of Y Chromosome (p437-8)

a. Few genes relative to X chromosome (~70 vs 1600)

b. Pseudoautosomal region (p437)

i. Location? % of chromosome?

ii. Recombination

iii. Large portion of Y genes

c. Male Specific Region (MSY) (p438)

i. Location? % of chromosome?

ii. Lots of heterochromatin, few genes

iii. Most genes assoc’d with testicular fxn (e.g. SRY, DAZ, USP9Y)

B. Co-Dominant Inheritance

1. Characteristics

a. Products of both alleles expressed in heterozygote (p438)

2. ABO Blood Type and MN antigens (Glycophorin A; GYPA) (p439)

a. Punnett square (p440)

II. Complicating Factors for Single-Gene (Mendelian) Traits

A. Clinical Vignette

B. Gene-gene Interactions

1. Epistasis: non-allelic gene-gene interactions that modify the expression of a trait (p444)

a. Manifests as either incomplete penetrance or variable expression

b. Causes of Epistasis

i. Modifying Loci (p450)

(a) α-thalassemia and Sickle Cell

ii. Genetic Background (p451)

2. Incomplete Penetrance: All-or-none pheno expression of (typically dominant) mut traits in heterozygotes (p445)

a. Incomplete vs Complete (p444)

b. Example (*curly-tail; CT*) (p446)

i. Expected phenotypic incidence vs epistatic effects (p446)

c. Pedigree example (p447)

i. Can mimic autosomal recessive inheritance, subtle differences

3. Variable Expression: Mut allele affects all individuals (i.e. penetrance = complete), but to different extents (e.g. severity, type of manifestation, age of onset) (p445)

a. *Hooded* Rats Example (p449)

i. What causes their differences? *Other loci in bkgrnd*

4. Variable Expressivity in Humans

a. Marfan Syndrome (p452)

i. Pedigree example

b. Neurofibromatosis (Neurofibromin; *NF1*) (p452)

c. Huntington’s Diseases (p453)

i. Delay of Onset

ii. Huntingtin (*HTT*)

III. Other Complicating Factors

A. Genetic Heterogeneity

1. Alleleic Heterogeneity

a. Diff muts occurring at *same locus* that cause diffs in “affected” phenotypes (p454)

i. Not due to epistasis

b. Example (Phenylketonuria; PKU) (p455)

i. Phenylalanine hydroxylase

c. Phenotypic heterogeneity (p456)

i. RET proto-oncogene

ii. Hirschprung disease and Multiple Endocrine Neoplasia (MEN)

2. Locus Heterogeneity

a. Muts at diff loci result in similar phenotype (p457)

b. Non-syndromic Deafness (*GBJ2*)

B. Environmental Factors (p458)

C. Sex Influences

1. Sex-Limited Traits (p459)

a. Expression of certain phenotypes only in one sex

b. Examples: Prostate CA, milk production.

2. Sex-Influenced Traits (p460)

a. Mode of trait’s expression modified by gender of affected individual

b. Examples

i. Male pattern baldness

(a) Dominant in males, recessive in females

ii. Hemochromatosis (p460)

D. Pleiotropy

1. Mutation in one gene has multiple, distinct manifestations in different tissues (p461)

2. Examples

a. CT (*curly-tail*) Trait

b. Phenylketonuria (PKU) (p461)

E. Phenocopy

1. “A condition where environmental influences result in a phenotype that mimics a genetic disease” (p463)

2. Thalidomide and Pseudothalidomide Syndrome (SC Phocomelia) (p463)

F. Mosaicism: Presense of 2+ genetically distinct cell lines in an individual (p464)

1. Somatic: Triggered by post-zygotic event.

2. Germline: Triggered by pre-zygotic event.

a. Can be responsible for occurrence of dominant phenotype when neither parent affected

i. Diagram (p465)

b. Duchene Muscular Dystrophy (DMD) (p465)

c. Recurrence risk, sporadic mutation (p466)

IV. Multifactorial and Polygenic Inheritance

A. Definitions

1. Multifactorial: “Phenotypic traits resulting from the interaction of multiple environmental factors with multiple genes.” (p647)

B. Multifactorial Inheritance

1. Characteristics

a. “Characterized by risk conditioned by the number of mutant genes inherited” (p470)

i. Risk (should) increase for sibs of pts showing severe expression of trait.

b. Does not demonstrate Mendelian patterns of inheritance (p471)

c. Does demonstrate Familial Aggregation (measured by relative risk)

2. Relative Risk Ratio (λr) reflects level of familial aggregation exhibited by a disease

a. Formula for Relative Risk Ratio (p471)

b. Rarer the disease, the more likely the aggregation is influenced by genetics and not due to coincidence (genetics p153)

3. Concordance/discordance (p472)

a. Relatives who share disease-predisposing alleles can be discordant for phenotype due to non-genetic factors (so what?)

b. Disease prevalence increases in close relatives to proband

c. Concordance in monozygotic vs dizygotic twins (which is greater?)

4. Curve of affected individuals vs age (p472)

C. Gene-Environment Interactions

1. α1-Anti-Trypsin (α1-AT; *SERPINA1*) (p468)

a. Function? *Inhibs trypsin & elastase*

b. Superoxide anion

c. Survival curves (p469)

2. Phenylketonuria (PKU) (p469)

D. Polygenic/Quantitative Inheritance

1. Variance and its Components (p473, genetics p156)

a. Environmental vs Genetic (which generations exhibit which?)

b. Corolla length in plants

c. VT = VE + VG (p474)

2. Heritability (p475, genetics p158)

o. “A measure of the extent to which different alleles at various loci are responsible for the variability in a given quantitative trait seen across a population.” (genetics p158)

i. Formula? (p475) *know the formula!*

a. Estimates of heritability for common disorders (p475)

b. Some Human traits have a continuous normal distribution (p476)

e.g. Blood pressure

c. Some disorders have multifactorial inheritance (p476)

e.g. Diabetes Mellitus, HTN

d. Increased VE → Decreased heritability (p492, but no more info given)

3. Twin Studies and Polygenic Traits

a. Dizygotic vs Monozygotic (genes in common? Enviro in common?) (p477)

i. Blood pressures studies in twins (showed what? How?)

b. Concordance in Polygenic Traits (p477)

i. “In MZ twins, likelihood of concordance <100% but much > than chance of same in DZ twins. Usually (in MZ) in range of 20-40%”

c. Correlation in Polygenic Traits (p478, genetics p157)

o. Correlation: “relationship b/n two factors (variables) for a given item or individual. These factors are often measured on a continuous scale” (corrected slide)

(a) Measured by Correlation Coefficient (R), where 1 = perfect correlation, and 0 = no correlation.

i. Parent-Child vs Adopted Child-Adopted Child in Blood Pressure (p478)

E. Models for Inheritance of Quantitative/Polygenic Traits

0. “Quantitative inheritance is polygenic” (p479)

1. Additive Inheritance Model: Approximates continuous distribution

a. Theoretical Mechanism and Assumptions

i. Locus A and B, plus and minus alleles (which is which?) (p480)

ii. Locus A and B, and each plus or minus allele exert equal effects (magnitude, not direction) on expression of the trait

b. Distribution and crosses (p481)

c. Selection for quantitative trait expression (p482)

d. Animal model: Blood Pressure (Dahl Salt-Sensitive and Salt-Resistant) (p483)

2. Threshold/Liability: Discontinuous distribution of phenotypes

a. Threshold Trait Characteristics

i. Quantitative traits expressed in limited # (usually 2) of phenotypes, but which are assumed to be based on continuous distribution of contributory factors for the trait (p484)

e.g. Cleft Lip/Palate (p485)

ii. Individuals → affected when “genetic predisposition is above certain (threshold) value” (p486)

(a) Normal distribution underlying phenotypes, and the threshold value are both based on Genetically-Determined Liability (at end of lec)

b. Risks

i. Recurrence risks represent avg risks, and will vary among diff families (p484)

ii. Risk increases c # of affected family members, and c severity of disorder

iii. Relative (differential) risk of relatives of proband increases as frequency of disorder in gen pop decreases (p485)

iv. If sex-ratio of affected progeny is significantly skewed, then offspring of affected probands of the *less frequently affected sex* have a higher relative risk.

c. Genetic and Environmental Components

i. Genetically-Determined Liability (p486)

(a) Defined as fxn of 1) # of mut alleles carried and/or 2) severity of effects assoc’d c the mut alleles

ii. Distribution of Genetically-Determined Liability for Threshold Traits (p487)

(a) Genetic component shift (for relatives of affected individuals) (p488)

(b) Environmental component shift (p488)

(c) Environmental shift in sex-influence traits (p489)

**Population Genetics and Risk Assessment** (p501-586)

I. Probability Basics

A. Mutually-Exclusive Events

1. Addition Rule (p506)

B. Successive Independent Events

0. Multiplication Rule (p508)

1. With Replacement (p506)

a. “At Least” Events (p509)

i. Method 1 (hard way) (p510)

ii. Method 2 (easy way) (p511)

2. Without Replacement (p508)

II. Population Genetics

A. Definitions

1. Allele Frequency (p512)

2. Genotype Frequency (p513)

3. Phenotype Frequency (p513)

B. Calculating Allele Frequencies (p513)

p + q = 1 where *f*A (frequency of A) = p, and *f*a = q

C. Calculating Genotype and Phenotype Frequencies (p515-6)

p = *f*A = [*f*AA + 0.5(*f*Aa)] and q = *f*a = [*f*aa + 0.5(*f*Aa)]

OR… you could just count them from a table.

D. For Three Alleles (p517)

p + q + r = 1 where each letter is the frequency of an allele

III. Hardy-Weinberg Equilibrium

A. Conditions Required to Maintain Equilibrium

1. Random Mating (p520)

2. Large Population (p521)

a. Fixation of Genes

b. Founder Effect (p522)

c. Bottle Neck

3. No Selection

a. Reproductive Fitness (p524)

b. Heterozygote Advantage

i. *Vkorc1* (p525)

ii. Other examples: *CFTR*, *HEXA*, *PAH*, *APOL1*

4. No Mutation

5. No Migration (in or out) (p526)

B. Proof of Hardy-Weinberg Law (p527)

1. Punnet square of random combination of gametes

C. Use of Hardy-Weinberg Law (p529)

(p + q)2 = (1)2 = p2 + 2pq + q2 where p2 = *f*AA, 2pq = *f*Aa, and q2 = *f*aa

1. Sample Calculations (p529-33) *DO THE SAMPLE PROBS!*

D. Special Case: X-linked Traits and Hardy-Weinberg Equilibrium

1. Differences arise from gene dosage diffs and sex chromosome diffs (p534)

a. Punnett square incorporating differences (see below)

2. Female Frequencies (p535)

a. Follow normal rules for two-allele frequencies

3. Male Frequencies (p535)

a. Genotype frequencies = Allelic frequencies (p + q = 1)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | X-Bearing Sperm | | Y-Bearing Sperm |
|  |  | XA (p) | Xa (q) | Y |
| Eggs | XA (p) | XAXA (p2) | XAXa (pq) | XAY (p) |
| Xa (q) | XAXa (pq) | XaXa (q2) | XaY (q) |

4. Sample Calculations (p536-8)

a. Assumptions for female phenotypic frequencies (p538)

IV. Mutation Selection Equilibrium

A. New alleles arise by (sporadic) mutation, and are maintained or removed by selection

B. Fitness and Coefficient of Selection (p539)

0. What are they? How are they related? What is their range?

1. Warfarin Resistance in Rodents Example (p540)

a. *Vkorc1* (Vitamin K Epoxide Reductase Complex Subunit 1)

b. Frequency of resistance allele ≠ 1.0, why? (p541)

C. Mutation Selection Equilibrium in Populations

1. With Autosomal Dominant Traits

a. Here, Fitness inversely assoc’d c proportion of affected pts from new mutations (p541)

i. Why?

b. To remain in Hardy-Weinberg Equlibrium: µ = s q or µ = (1-f) q (p542)

2. With Autosomal Recessive Traits (p543)

a. Selection is much less influential on allele frequencies (why?)

3. With X-linked Recessive Traits (p543-4)

a. If affected phenotype is “benign”: µ = s (q/3) (why?)

b. If affected phenotype is lethal: µ = s q where s ≈ 1 (meaning?)

V. Inbreeding

A. Consanguineous Mating (p545)

1. Progeny are considered “inbred”

B. Coefficient of Relationship

1. Measure of proportion of genes shared by two related individuals (p546)

a. Equals the probability that a particular allele is shared (p547)

2. Formula CoR = (1/2)n where n = # matings connecting the pair (p546)

a. Diagram to find degree/number of connecting matings

C. Coefficient of Inbreeding (F)

1. Measure of the probability that a homozyg has received both alleles from a common (recent) ancestor AND the proportion of loci that are homozygous, i.e. for which both alleles are identical-by-descent (p547)

2. Formula F = (1/2)n+1 = (1/2) x CoR where n = # matings connecting (p548)

a. It’s the P(inheriting common allele) x P(other parent also passes on that allele)

b. Only applies for low levels of inbreeding (i.e. not livestock or lab animals)

D. Risk of Inheritance of Recessive Traits in Inbreeding

1. Est that most individuals carry 2-6 recessive lethal muts, and 1 autosomal recessive mut for harmful but viable disorder (p549)

E. Inbred Strains (p550)

1. 20+ gens of brother-sister mating → 99% of loci are homozygous

VI. Risk Assessment

A. Clinical Vignette (p550)

1. Events in probability calculations are EITHER mutually exclusive OR independent (p552)

2. Sex of offspring is an independent event (p553) *KNOW THIS!!*

B. Bayesian Analysis (p553, see sample prob p555 for calc)

1. Prior Probability

2. Conditional Probability

3. Joint Probability

4. Posterior (Relative) Probability

C. Bayesian Analysis with Dominant Trait

1. Delayed Onset of Disease (p554)

a. Prior probability of being heterozygous is modified by proportion of heterozygotes showing symptoms (at a given age)

b. Sample Calculation c Huntington’s (p555)

2. Graphic Explanation (p555-6)

D. Bayesian Analysis with Recessive Trait (p557)

1. P(unrelated spouse is a carrier)

2. Probability of having carrier progeny out of unaffected progeny (p558)

3. Identifying Obligate Heterozygotes (p559-60)

VII. Summary of Steps in Solving Genetic Problems

A. Autosomal Recessive (p562)

B. Autosomal Dominant (p564)

C. X-Linked Recessive (p564)

D. X-Linked Dominant (p565)

VIII. Practice Problems (p568-end)

**Recombinant DNA Technology** (p587-613)

I. Overview (p591)

A. Gene therapy: insertion of a gene (or other genetic element) into an organism which corrects a genetic defect.

B. SCIDS (Severe Combined Immune Deficiency Syndrome) (p592)

1. Adenosine Deaminase (ADA), Allogenic Bone Marrow Transplant, Polyethylene glycol.

2. Amplification/expression of ADA in prokaryotic vectors (p608)

3. Verification of ADA expression from vectors (p611)

II. cDNA Synthesis

A. Reverse Transcriptase (p593)

1. mRNA → cDNA

2. Oligo(dT) DNA primer

B. PCR (p593)

C. Overall mechanism (p594)

1. Restriction Endonucleases (*EcoRI, PstI, HaeIII*) (p595)

a. Blunt vs Sticky ends (specificity and efficiency?)

D. Genomic and cDNA Libraries (which vectors are used?) (p603)

III. Cloning Vectors

0. DNA insert sizes: BAC > Phage > Plasmid (p607)

A. Plasmids

1. Overview (p596)

a. Vector of choice for → bacterium

b. Can replicate independently from host

c. Strandedness/composition? (i.e. ds, ss, DNA, RNA?)

2. pGEM-4 (p597)

a. 3 key elements: Selectable marker (what?), Ori, Polylinker

3. Recombinant plasmid construction (p598)

a. T4 DNA Ligase

4. Plasmid Transformation and Selection (p599)

5. Verification of Recombinant Plasmids (how does each of these verify? p599 for answers)

a. Restriction digestion (p600)

b. Southern blotting

c. DNA Sequencing (p600-2)

i. Sanger Sequencing

B. Bacteriophages (bacteriophage lambda)

1. Overview (p603)

a. Relatively efficient for DNA insertion.

b. Usually cause lysis of bacteria.

c. “Plaques”

d. Lytic phase of bacteriophage lambda life cycle (p604)

e. Phage aren’t used as much anymore for amplifications (p606)

2. cDNA Library construction with bacteriophages (p604)

a. Each cDNA → own phage particle

b. T4 DNA Ligase

c. Same process for genomic library (p606)

3. Screening Methods from Plaques (p606)

a. Immunoblot for cDNA, Southern hybridization for cDNA or genomic

C. Bacterial Autonomous Chromosomes (BACs)

1. Overview (p607)

a. For cloning large DNA segments (>100kb)

i. Human Genome Project used these a lot

b. Highly stable and maintained as separate chromosome in host

c. Based on *E. coli* fertility plasmid (F- plasmid)

i. Genes for partitioning plasmid to daughter cells

ii. Selection marker and Ori

IV. Expression of Cloned Genes

A. In Prokaryotes

1. Construction of Insert (diagram p608)

a. *E. coli* promotor (strong and inducible) (where relative to insert? why inducible?) (p608)

b. Ribosome binding site, termination signals (where relative to insert?)

c. A cDNA of the protein of interest must be used if it’s euk. (why?)

2. Proinsulin synthesis and Humulin (p609)

B. In Eukaryotes

1. For “large scale production of proteins and peptides of therapeutic importance, especially when specific post-translational modifications are required for protein function” (p610)

a. “Human gene therapy vectors are eukaryotic expression vectors” *italics in lec*

2. Construction of Insert (diagram p610)

a. Inducible promoter

b. Termination signals

c. cDNA or genomic

d. Appropriate cell type is necessary as host to ensure proper post-transl mods

V. Western/Immunoblot Analysis

A. 1° vs 2° antibodies (p611)

B. Filter membrane

C. Not “hybridization”

**Mitochondrial Disorders** (p614-645)

I. Characteristics of Mitochondria

A. Clinical Vignette (p616-20)

B. Oxidative Phosphorylation and ATP Generation (p621)

1. Summary of Electron Transport Chain (p623)

C. Mitochondrial Structures (p622)

II. Characteristics of Mitochondrial Genome

A. Size and number of genes

1. Many genes involved in mitochondrial fxn

a. For ETC proteins: 80+ from nucleus, 13 from mtDNA (p623)

i. Genes for Complexes 1, 3, 4, and 5. Not 2, not Coenzyme Q.

b. General proteins: (p624)

i. mtDNA: 13 polypeptides (for OxPhos), 2 rRNAs, 22 tRNAs

ii. ~ 1000 proteins from nucleus

2. mtDNA = circular dsDNA ~16kb long (p624)

a. Replicated, transcribed, translated all within mitochondrion

B. Ploidy (p625)

1. 2-10 copies of mtDNA per mitochondrion. 100s-1000s mitochondria per cell.

a. Useful for forensic/anthro investigations

C. Unique Characteristics of mtDNA

1. Unique genetic code (p625)

2. 12S and 16S rRNA (how is this unique?)

3. Continuous transcription of multiple genes (some polycistronic)

a. No introns

4. Endosymbiont Theory

5. Very high mutation rate (7-10x nuclear DNA rate) (p626)

a. Can change within single generation via somatic mutations

b. Mitochondrial DNA Polymerase γ (PolG, D257A) (p626-7)

i. Experimental mutation in mice (symptoms? Effect on mutations and survival?)

ii. Endurance training (p628)

III. Maternal Inheritance of Mitochondrally-encoded Traits

A. Mother → Son/Daughter; Sex bias in transmission, but no sex bias in progeny (p628)

1. Sperm vs Egg mitochondria?

2. Uniparental transmission (discussed in different lecture)

B. Paternal transmission technically possible, but extremely improbable (p629)

1. Autophagosomes

C. Grid to characterize inheritance (p638, or p32 of this outline)

IV. Characteristics of Mitochondrial DNA Affecting Phenotypic Expression

A. Elevated Mutation Rate (p626)

B. Homoplasmy vs Heteroplasmy (p629)

1. ~99.9% of mtDNA in *normal* individual is identical

2. Effect of age?

C. Replicative Segregation (p630)

1. Autonomous replication

2. Independent segregation (no spindles) (can lead to what?)

D. Tissue Specific Differences (p631)

1. OxPhos

2. Which tissues most heavily dependent? *Heart, SkM, brain, CNS*

3. Other heavily dependent tissues: liver, kidney, pancreatic β-cells

4. Effect of tissue specific differences on disease?

E. Threshold Effects (p632)

F. Decline of Oxidative Phosphorylation with Age

G. Summary

1. More than just mtDNA problems that cause mitochondrial diseases (what else?)

V. Mitochondrial Diseases

A. Deletions

1. “Neither size nor position of deletion are well-correlated c either enzyme deficiency or severity of disease” (p633)

2. Mitochondrial Myopathy (MM) (p633)

3. Kearns-Sayre Syndrome (KS) (p634)

4. Occasionally assoc’d c Diabetes and Deafness (p634)

B. Point Mutations

1. Protein-encoding Genes

a. Leber’s Hereditary Optic Neuropathy (LHON) (p635)

2. tRNA-encoding Genes (p636)

a. Myoclonic Epilepsy with Ragged Red Fiber Disease (MERRF)

b. Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Symptoms (MELAS)

c. Maternal Myopathy and Cardiomyopathy (MMC)

VI. Handout and Practice Questions (p639-43)

**Linkage Analysis** (p644-702)

I. Linkage Analysis in Experimental Populations

A. Two Loci on One Chromosome

1. Backcross double heterozygote to double recessive (p647)

a. [Pale ears, Rubi eyes] x [Normal, normal] → ER || er

b. Schematic of Backcross (p652)

i. Expected gamete frequencies?

ii. Why use double recessive parent? (p653)

B. Crossover Events can produce mixed heterozygocity gametes in linked alleles (p648)

C. Types of Gametes (p648)

1. Parental: chromosome c same genotype as either of parental chromosomes

2. Recombinant: chrom c different genotype from either parental chrom

a. Chiasmata (diagram p649)

i. Multiple crossing over events possible b/n homologous chromosomes

D. Recombination Frequency

1. “Proportion of crossover events and therefore the proportion of recombinant gametes is a function of the distance b/n the 2 loci on a chromosome” (p650)

2. Recombination Frequency/Ratio (RF or θ) (p652)

a. RF in Pale/Rubi mouse backcross (p653)

b. Typical results for RF depending on linkage in 100 Meioses (p654)

i. Diagram of recombinations

c. RF or θ = [(# recombinant haplotypes) / (# recomb + parental haplotypes)] (p665)

i. Range b/n 0 (complete linkage/no recombination) → 0.5 (unlinked)

E. Linkage Mapping

1. High frequency of crossover can distort (i.e. lengthen) distances b/n loci. Opposite too. (p651)

2. Genetic Map Unit, centiMorgan (cM) (p652)

a. ~1-2 x 106 bp (though technically can’t be converted to a distance) (p651)

b. Relationship with θ (p665)

3. Determining order of loci from linkage mapping distances (p655)

II. Linkage Analysis in Humans

A. Haplotype and Linkage Disequilibrium

1. Polymorphic markers can be linked to diseases, requires study of large families (p656)

2. Haplotype (p665)

3. Linkage disequilibrium: the non-random association of a trait with a particular haplotype (p682)

4. Example (Autosomal dominant) (p657-8)

5. Example (Haplotype analysis) (p658-663) *DO THESE!*

6. Pseudohypoaldosteronism Type II (p663)

a. No information given abt disease, just shows an example pedigree.

B. Lod Scores and Maximum Likelihood Method

1. Likelihood Ratio (Odds Ratio): Probability that two markers (or loci) are linked (p664)

2. Logarithm of Odds Ratio (Lod Score, Z): log10(Likelihood Ratio) (p664)

a. Z = log10(Likelihood of linkage/likelihood of no linkage)

b. Z = log10 Likelihood of observed data at specified θ (p666)

Likelihood of observed data at θ = 0.5)

c. Lod scores for a particular trait from diff families can be combined (p667)

i. This is the major advantage of using Lod scores *KNOW!!*

3. Maximum Likelihood Ratio

a. Determined by finding the Lod scores for many values of θ from 0 → 0.5 (i.e. finding the Lod scores at different distances) (p666)

b. Highest Lod Score is considered best estimate of θ (p667)

i. Lod > +3.0 means linkage

ii. Lod < -2.0 means no linkage

iii. What does between mean?

C. Lod Score Calculation Example (Autosomal Dominant)

0. Basic Example (p668-72, in just text form p689)

1. Example: Complete Concordance (p672-3)

2. With Recombination (p673-4)

D. Inter-Family Comparison of Lod Scores (p675-7)

E. Comparison of Linkage and Cytogenetic Maps (p677)

III. Linkage and Quantitative Traits

A. Quantitative Trait Locus (QTL): “A chromosomal region containing a gene (or genes) that affect the phenotypic expression level of a quantitative trait” (p678)

1. Crossing over c QTLs

B. Mapping QTLs (p679)

C. Genome Wide Association Studies (GWAS) (p679)

1. Case-control or cohort studies

IV. Practice Questions (p693-702)

**Dynamic Mutations, Uniparental Disomy, Genomic Imprinting** (p703-754)

I. Dynamic Mutations – Triplet Repeats

A. Properties of Triplet Repeat Expansions and Disorders

1. Stem from expansions of trinucleotide repeat or minisatellite DNA regions located within genes or in regulatory regions. (p710)

a. Can occur within any gene or gene regulatory region (p707)

i. 5’-UTR (Fragile X), introns, exons (Huntington), 3’-UTR

ii. DNA sequence repeat normally polymorphic in copy number though (p711)

b. May have interspersed sequences that differ from repeated sequence (p711)

i. May have stabilizing effects; prevent polymerase slippage

c. # of repeats is unstable (varies by individual, and by pedigree) (p710-1)

2. Affected individuals have sudden increase in # of repeats generated during gametogenesis (p710)

a. Triplet expansion can alter either expression or structure of a protein

b. Pre-mutations (p710)

c. Genetic Anticipation (Sherman Paradox) (p709)

i. 2 effects produced by Genetic Anticipation?

ii. Parent of Origin Effects

3. Differences that change expression of disorders (p712)

a. Composition and length of repeat motif

b. Location of repeat c respect to RNA transcript

c. Mechanism of action (gain or loss of fxn)

i. PolyQ (GOF), RNA toxicity (GOF), hypermethylation of promoter

d. Inheritance patterns

B. Mechanism – 3 General Mechanisms (p712)

1. Slippage during DNA replication (diagram p713)

a. Can’t occur w/o repeated sequences already present

2. Misalignment with subsequent excision repair (p712)

3. Unequal crossing over and recombination

4. “Both nucleosome assembly and chromatin strx may affect pathogenesis” (p712)

C. Consequences of Triplet Repeat Expansion

1. Loss of Function (p713)

e.g. Fragile X and hypermethylation

2. Gain of Function (p714)

e.g. Huntington Disease and polyQ tract

X. Fragile X Syndrome

1. Clinical Aspects (p714-5, all added to diseases table)

o. X-linked dominant

a. Number of repeats for normal, premutation, full mutation? (p717)

b. Carriers of premutation affected in degrees (p718)

i. Three mild clinical disorders found (what are they?)

c. Exhibits Genetic Anticipation (p709)

2. CGG expansion → hypermethylation of *FMR1* → Loss of function bc no FMRP made (p713)

a. Function of *FMR1* and FMRP (p716)

i. Polyribosomes, translation-competent mRNP

ii. FMRP-mRNP complex, Synaptic transmission (p717)

iii. 5’-UTR (CpG Island) (p718)

b. Folate-sensitive fragile site (p715)

c. Extensive repeat number mosaicism (what and why?) (p718)

3. Inheritance (p718)

a. Maternal premut → higher risk of full-mut in progeny than from paternal premut

b. Size of premut (maternal meiosis): <70 repeats : 20% risk. >90 repeats : >99% risk.

4. Testing

a. Criteria (p719)

i. Basically anyone c MR/dev delay of unknown cause, anyone c fam hx, and anyone c physical features suggestive of it.

b. BssHII restriction endonuclease (p719)

X. Huntington Disease

1. Clinical Aspects (in diseases table)

o. Autosomal dominant

a. Choreioform movement (p720)

b. Neuropathology: Basal ganglia and lateral ventricles (p721)

b. Paternal meiotic instability → higher risk of expansion in paternal alleles (p722)

c. Number of repeats and subset c reduced penetrance

2. CAG expansion → polyglutamine (PolyQ) tract → abnormal protein aggregation (p714)

a. Aggregations clog nerve-cell fxns, can also lead to apoptosis or autophagy

i. HAP1, Huntingtin (p721)

II. Uniparental Disomy

A. UPD: When child inherits two copies of a chrom from one parent, and none from the other (p723)

1. Maternal vs Paternal Uniparental Disomy (p724)

2. Clinical Significance of UPD (p731)

a. Imprinting effect → Prader-Willi and Angelman

b. Risk of recessive disease → Cystic Fibrosis and Transient neonatal diabetes

c. UPD can also be benign (p724)

B. Mechanisms for UPD formation: all require 2 consecutive mistakes

0. Meiotic nondisjunction → Trisomic zygotes

a. Meiosis I vs Meiosis II (gametes formed?) (p724)

i. Disomic cell, Nullisomic cell

b. Fertilization following nondisjunction in meiosis (p725)

i. Trisomic zygote (diff b/n meiosis I vs meiosis II zygote?)

ii. Majority of trisomic zygotes spontaneously abort or miscarry, but not chrom 13, 18, or 21. (p726)

1. Trisomic Rescue (p727-8)

a. Probabilities of results? (p727) *2/3 normal, 1/3 UPD*

2. Monosomic Rescue (p728)

a. Duplication of a chromosome from a monosomic zygote

3. Gamete Complementation (p728)

C. Types of UPD (diagrams p729)

1. Uniparental Heterodisomy: “Inheritance of two homologous (i.e. nonidentical) chrom from one parent”

a. Error in meiosis I followed by trisomic rescue

b. Anaphase lag (p729)

2. Uniparental Isodisomy: Inheritance of “two identical copies of a single chrom” from one parent (p729)

a. Error in meiosis II, fertilization, then followed by trisomic rescue

3. Sample gel electrophoresis runs of heterodisomy and isodisomy (p730)

III. Genomic Imprinting

A. Overview

1. Of 2 copies of genes we inherit from parents, both usually “turned on”, but sometimes one is inactive (p731)

a. Some only active when inherited from mother, others only from father (p732)

2. Imprinting: Epigenetic marking of a gene based on its parental origin (p732)

a. Involves at least 150 genes in humans; is normal mechanism for gene regulation (p732)

b. “Imprinted allele suppressed in some (or all) somatic tissues in embryo. Can continue into adulthood.”

c. Epigenetic: Is heritable through cell divisions, but is not an alteration to 1° DNA sequence (p732)

B. Characteristics

1. Imprinted genes usually occur in clusters

a. Imprinting Centers: “small regions (usually adjacent to the imprinted gene cluster) that regulated expression within that region” (p733)

i. No common sequence motif

ii. lncRNAs often involved

2. Monoallelic expression despite biallelic transmission (p733)

a. A form of “Uniparental transmission”

3. Imprinting markings are reversible (p733)

C. Mechanisms of Imprinting

1. DNA Methylation of Cytosine residues in promoter regions (p734)

a. Imprinting factor

2. Histone Acetylation/Deacetylation

a. Acetylation → active (open) chromatin (p734)

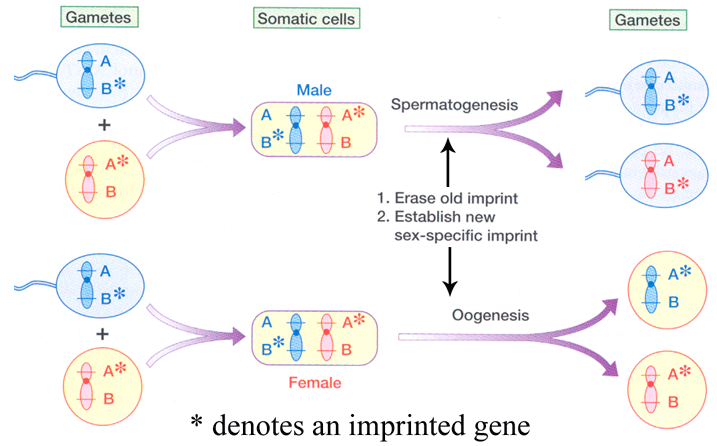
b. Deacetylation → Inactive (condensed) chromatin

3. Methylation and Deacetylation relationship (p735)

a. DNA Methyltransferase

b. HP1 (heterochromatinizing protein)

D. Establishment of Genomic Imprinting (p735)



1. During gametogenesis, *de novo* methylation occurs earlier in spermatogenesis than it does in oogenesis. (p736)

E. Inheritance Patterns of Genomic Imprinting (summary p738)

1. Paternal vs Maternal Imprinting (p736)

a. Paternal Imprinting: “phenotypic effects of an allele are not expressed (silenced) when inherited from father.”

b. Maternal Imprinting: “phenotypic effects of an allele are not expressed (silenced) when inherited from mother.”

i. Pedigree of maternal imprinting with autosomal dominant mutation (p737)

c. They produce opposite patterns of inheritance (diagram p737)

i. Opposite patterns of expression for knock-out β-galactosidase in mice (p740)

(a) *Grb10* (growth-factor receptor-bound protein 10)

2. No sex bias in progeny or transmission, but sex bias in expression (p738)

a. Pattern very similar to autosomal inheritance with incomplete penetrance

F. Hypotheses of Evolutionary Functions for Imprinting (p752)

1. Genetic competition between paternal and maternal chromosomes

a. Male genome favors growth enhancement

b. Female genome favors growth inhibition (easier to deliver)

2. Host-defense mechanism akin to methylation of foreign DNA

4. Suppressive regulation of chromosomal aneuploidy to control malignant clonal developments in tissues.

5. To conserve some traits inherited from one parent only.

IV. Genomic Imprinting and Uniparental Disomy in Disease

0. Tables of diseases and their imprinted regions (p739)

A. Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS)

1. Mechanisms same for both (p742)

a. UPD, imprinting errors on long arm of chrom 15, or microdeletions within 15q11-q13

b. Results in loss of expression of “key gene” usually expressed from an allele present on a single chrom (the other is imprinted) (p742)

i. Both copies of gene can also be silenced via imprinting (either via UPD or imprinting error)

2. Prader Willi Syndrome

a. Symptoms (p743)

b. Percentages for each genetic basis of the disease (p743)

i. Diagram p747

c. Molecular Etiology of PWS

i. Bc maternal UPD or paternal deletion causes PWS, suggests that paternal alleles are usually active and the lack of activity is the source of the disease (p748)

(a) SNURF-SNRPN locus *proteins* are not candidates for PWS

i. HBII-85 snoRNA (small nucleolar RNA) gene cluster implicated in PWS (p749)

(a) 1st example of disease from non-coding RNAs

(b) Located in C/D box of 3’-UTR of SNURF-SNRPN locus

(c) C/D box snoRNAs typically pair c rRNA or snRNA and methylate specific nucleotides, however, fxn of this one is unknown (p750)

3. Angelman Syndrome (AS)

a. Symptoms (p744)

b. Percentages for each genetic basis of the disease (p745)

i. Diagram p748

c. Molecular Etiology of AS

i. Maternal deletion, paternal UPD are causes, which suggests maternal allele is responsible for normal fxn. (p750)

ii. *UBE3A* (ubiquitin protein ligase E3A) is candidate gene. (p751)

(a) Transfers ubiquitin to protein substrates in ubiquitin-proteosome proteolytic pathway; brain-specific imprinting

iii. Unsilencing of paternal imprinted *UBE3A* in mice by Topotecan (p751)

(a) *UBE3A*-antisense transcript overlap

4. Testing for PWS/AS

a. Bisulfite treatment converts C→U in unmethylated regions.

i. New U are converted to T via PCR amplification (p746)

b. SNRPN polypeptide (SmN) (p744, 46)

c. FISH (fluorescence in situ hybridization) (p747)

**Gene Therapy** (p755-787)

I. Overview

A. Germ Line Gene Therapy (p756)

1. Ethics?

2. Transgenic Animals (p757)

B. Somatic Gene Therapy (p758)

1. *Ex vivo* vs *In vivo*

2. Ethics?

C. Monogenic treatments vs polygenic treatments (p757)

1. Targets for therapy?

2. Monogenic disorders easier to treat (why?)

D. “Most challenging aspect of gene therapy is efficient delivery and subsequent expression of the therapeutic gene” (p759)

II. Gene Delivery Vectors

A. Non-viral Vectors

1. Pros vs Cons (p759)

a. Pros: minimal host immune response, cost-effective for producing large amts

b. Cons: inefficient gene delivery, no vectors thus far delivered have → permanent changes.

2. Eukaryotic Expression Vectors (i.e. Plasmids)

a. Structural diagram (p760)

i. 3 critical parts of a plasmid?

b. Constructed on prokaryotic plasmids in bacteria, then transferred to euk cells (p760)

c. Directly injected → target tissues

d. Cationic Liposomes (Lipofection) = most common/efficient for plasmids

i. Mechanism of construction and transferal (p761)

e. Very large doses required (1016 copies per dose)

B. Viral Vectors

1. Pros vs Cons (p762)

a. Pros: high efficiency, some can permanently change host genomes

b. Cons: Elicit immune response (sometimes), labor-intensive for large quantities, based on an infectious agent and can have unintended problems

2. Viral Genetic Material

a. “Packaging signals” and Ori, Replication and regulatory genes, Structural genes (p763)

b. Generic viral replication cycle

i. Virus receptors, Provirus

ii. Assembly, not all viruses → lysis (p764)

c. Complementation (p764-5)

3. Viral Vector Production

a. Insertion of gene-of-interest (how is this related to complementation?) (p765)

b. Assembly on plasmids (which genes on which plasmids?) (p766)

c. Eukaryotic “Packaging cells” (p766)

i. “Packaged” gene therapy vectors (i.e. viruses) won’t self-propagate (p767)

4. Retrovirus Vectors (p769)

a. Murine leukemia

b. Integrates into host genome

c. High # of recombinants produced

d. Potential to be contaminated c replication-competent virus → bad.

e. Potential for insertion into endogenous genes (e.g. oncogenes) → also bad.

5. Adeno-associated Virus Vectors (AAV) (p774)

a. *Parvovirus*

b. Nonpathogenic (at least seems to be)

6. Adenovirus Vectors (p778)

a. Non-integrative (episome)

i. Host cell division not required for transgene expression

b. High titers, 1011/ml

c. Infects wide variety of cell types

i. Can be engineered to be replication defective

d. Highly immunogenic, infects dendritic cells.

i. Normal human pathogen (circulating antibodies)

ii. Jesse Gelsinger (p782)

(a) Ornithine Transcarbamylase Deficiency, OTC gene

III. Monogenic Diseases

A. Severe Combined Immune Deficiency Syndrome (SCIDS; ADA Deficiency)

1. Disease review (p767)

a. ADA, deoxyadenosine, dATP, inosine

b. Treatments: PEG-ADA enzyme, gene therapy, allogenic bone marrow transplantation

i. allogenic BMT is most effective treatment; 1st line and GT used if no match.

c. Lymphocytes c ADA outgrow deficient lymphocytes

2. Retrovirus gene therapy for SCIDS (p769-70)

a. Reverse transcriptase, PBL or bone marrow, stem cell (p770)

b. Not perfect initially, but now much improved (p771)

c. SCID-X1

i. *LMO2*, c-onc genes, childhood T cell acute leukemia (p772)

B. Blindness (Leber’s Congenital Amaurosis)

1. Disease characteristics (p773-5)

a. *RPE65*, amaurosis

2. Adeno-associated virus gene therapy

a. Vitrectomy, subretinal cannula

b. Phase 1 trials: success but advanced symptoms more difficult to treat (p776)

IV. Polygenic Diseases

A. Cancer

1. Overview (p777)

a. Polygenic! Thus, target for gene therapy is contributory mutations

i. Therapies would require treatment of large number of tumor cells to be effective

ii. Temporary expression of the vector gene may be enough to kill the tumor

b. Gene therapy for CA must be better than chemo for it to be used.

c. Targeting tumor cells (p786)

i. Intratumoral injection

ii. Preferential infxn of proliferating cells

iii. Bystander effect (later in outline)

2. Therapeutic Strategies (p777)

a. Pro-drug activating enzymes (nontoxic prodrug → toxic metabolite) e.g. HSVtk

b. Replacement of defective tumor suppressor genes e.g. p53

c. “antisense” genes or microRNAs

d. Immune response stimulation (cytokine, T-cell receptor, MHC)

3. p53 Tumor Suppressor Gene

a. Most frequently mutated gene in multiple types of CA (p778)

i. Normally fxns as transcription factor

b. Restoration of gene can… (p778)

i. → apoptosis

ii. Can confer sensitivity to DNA damaging agents.

iii. Inhibit angiogenesis

c. Adenovirus Gene Therapy for p53 (p779)

i. Early and late genes, E1, E3 (which gene → packaging cell line?)

ii. Intratumoral injection

4. Herpes Simplex Virus Thymidine Kinase (HSVtk)

a. Protein is analog of cellular thymidine kinase (p782)

i. Lower specificity; phosphorylates acycloguanosine (Acyclovir)

ii. Mechanism of action (p783)

b. “Suicide” gene therapy (p784)

c. Action enhanced by Bystander Effect (p784)

i. Gap junctions (p785)

**Molecular Basis of Common Diseases** (p788-829)

I. Cystic Fibrosis (p791)

See Diseases spreadsheet (cell 55)

II. Hereditary Hemochromatosis (p800)

See Diseases spreadsheet (cell 56)

III. Factor V Leiden Thrombophilia (p807)

A. Incomplete Dominance (p813)

See Diseases spreadsheet (cell 57)

IV. Duchenne Muscular Dystrophy and Becker Muscular Dystrophy (p815)

See Diseases spreadsheet (cell 58-9)

V. Osteogenesis Imperfecta (p821)

A. Dominant Negative Mutations (p822)

See Diseases spreadsheet (cell 60)

**Cancer Genetics** (p830-858)

I. Definitions

A. Neoplasia (neoplasm), Cancer, Metastasis, Benign Tumor (p831)

B. Hyperplasia, Dysplasia, Cancer in situ (p831)

C. 3 main forms of CA (which basic tissue types involved in which? Which specific tissues?) (p832)

1. Sarcomas

2. Carcinomas (most common)

3. Hematopoietic and Lymphoid

II. Genetics

A. Hereditary Cancer Syndromes vs Sporadic Cancer (which is more frequent?) (p832)

1. Hereditary CA muts more common in TSGs than in oncogenes (p857)

B. Oncogenes (Proto-oncogenes) (what do they typically encode?) (p833) *list of typical products*

1. Dominant effect at cellular lvl (p834) *is an L.O.!*

a. Very few oncogenic mutations are inherited (why?)

C. Tumor-suppressor Genes (TSGs)

0. Recessive at cellular lvl (why? what mechs result in expression of pheno?)

a. Can show dominant pattern of familial inheritance (p834)

1. Gatekeeper TSGs (what do they typically encode?) (p833)

a. Examples, gene products, and disorders (p842)

i. *RB1, TP53*

2. Caretaker TSGs (what do they typically encode?) (p834)

a. Examples, gene products, and disorders (p843)

i. *BRCA1, BRCA2, MLH1, MLH2*

III. Tumor Progression

A. Hallmarks of Cancer – 6 Categories (p835)

1. Angiogenesis, self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, unlimited replication potential, tissue invasion/metastasis

B. Oncogenes and TSGs in tumor progression (p835)

1. Oncogenes and anti-apoptotic genes are activated or overexpressed

2. TSGs and apoptotic genes are inactivated

IV. Study of Cancer

A. Rous Sarcoma Virus (p836)

1. Fine-pore filter

B. Viruses used in labs to study experimentally reproducible cancers and ID genes.

1. Amplification for study (p837)

a. Loss of contact inhibition

2. Isolation of human oncogenes (p838)

a. Phage library, *Alu* probe

b. *Ras* oncogene was 1st oncogene ID’d this way.

C. Many human oncogenes originally ID’d from their isolated animal viral counterparts (p842)

V. Mechanisms of Cancer

A. Activation of Proto-oncogenes

1. Wide variety of proto-onc genes in diff parts of signal transduction can → CA (p840)

2. Genetic mutations that can convert proto-onc → oncogenes (p840)

a. Pt muts (e.g. *Ras*)

b. Dels of regulatory domains (e.g. Receptor Tyrosine Kinases)

c. Chrom translocations → novel fusion proteins (e.g. Bcr-Abl)

d. Chrom translocations → new strong promoter for proto-onc (e.g. Bcl2 in follicular lymphoma) *know this one! Only mentioned here, but listed in L.O.*

e. Gene amplification → overexpression (e.g. transcription factor N-Myc, the RTK in Her-2 breast CA)

B. *Ras* Oncogene (p839)

1. Encodes G protein

a. Intrinsic GTPase activity (how does this affect normal fxn?)

2. Point mut (G12V) constitutively activates Ras G protein (leading to what?) (p839)

C. *BCR-ABL* Translocation (p841)

1. Philadelphia chromosome

2. Chronic Myleogenous Leukemia (CML) (p841)

a. Imatinib (Gleevec)

3. BCR-ABL fusion protein → unregulated protein kinase activity → cell proliferation (p841)

D. *RB1* and Retinoblastoma

1. Rb function, E2F (what is the effect of phosphorylation?) (p843)

2. Retinoblastoma

a. Two-hit hypothesis (p844)

i. Unilateral vs bilateral cases (kinetics showed what?)

b. Mitotic Recombination (p845)

i. Loss of Heterozygosity *be able to define this! L.O.*

c. Inherited vs Sporadic (which one is uni vs bilateral? Relative frequencies?) (p846)

i. Sample pedigrees

E. p53 Tumor Suppressor Gene

1. Very commonly mutated in CAs (~50% of human tumors) (p847)

a. Majority are missense, interfere c functional domains

b. Dysfxn → accumulation of DNA damage (p848)

2. Mechanism of action (what does it lead to?) (p847)

a. ATM

b. Stress signals (p848)

3. Li Fraumeni Syndrome (LFS) (p849)

F. Familial Colon Cancer – 2 Major Types

1. Familial Adenomatous Polyposis (FAP) (see diseases spreadsheet)

a. Adenomatous Polyposis Coli gene (*APC*) (p849)

b. β-Catenin (p850)

2. Hereditary Nonpolyposis Colon Cancer (HNPCC)

a. MSH2, MLH1 (what do they do?) (p849)

b. Microsatellite instability (p851)

G. *BRCA1* in Breast Cancer

1. 5-10% Breast CAs occur in person c inherited predisposition (p852)

2. *BRCA1* (p853)

a. Very large gene

b. Somatic mutations aren’t found in *BRCA1* in sporadic CAs

i. Thus doesn’t follow 2 hit hypothesis

ii. Only inherited muts in the gene impart CA risk

c. Encodes protein involved in DNA damage response, and is phosphorylated by ATM protein.

3. Somatic Mutations in Breast Cancer (p854)

a. Her2/neu/c-ERBB2 receptor tyrosine kinase = growth factor receptor *L.O.!*

i. Target of drug (Herceptin)

b. Ras, c-myc, p53, Rb1 all frequently mut in breast CA

4. Microarrays for CA gene expression profiling (p854)

a. Study compiled info, found common trends (p855)

b. Found Estrogen Receptor positive (ER+) breast CAs had better prognosis (p855)

i. ER+ CAs dependent on estrogen for growth (p856)

ii. Tamoxifen = anti-estrogen, but eventually CAs → resistant

c. Prostate Cancer

i. Androgen receptor and testosterone synthesis (p856)

ii. Androgen-independent prostate cancer tumors

H. *Myc*

1. Gene for transcription factor (“strong proliferation signal”), overexpression can → CA if unchecked (p840, 2)

2. Normally, overexpression of *myc* → apoptosis via p53 TSG (p848)

3. *Myc* transcription can be activated by β-Catenin (p850)

**Pharmacogenetics** (p859-896)

I. Definitions

A. Pharmacogenetics (p863)

B. Pharmacogenomics

C. Ecogenetics

D. Pharmacokinetics: The effects of differences on rates of drug absorption, distribution, metabolism, and elimination. (p866)

E. Pharmacodynamics: The effects of differences on mechanisms of drug-target interaction and subsequent cellular and systemic results. (p866)

II. Adverse Drug Reactions

A. Incidence of ADR (overall) = 6.7%, incidence of fatal ADRs = 0.32% (p864)

B. Table of Commonly Identified Drugs Having Adverse Reactions

III. Population Drug Response Frequency Distributions

A. Unimodal: 1 maximum (p865)

1. “Indicate involvement of many factors, each c smaller effect on drug response” than factor in bimodal.

B. Bimodal: 2 maximums (p865)

1. “Indicate that a single factor has major impact on drug response” (e.g. a single gene)

2. Rare, but easier to ID than unimodal.

C. Trimodal: 3 maximums (p865)

1. “1:2:1 ratio”, means single locus, 2 possible alleles with incomplete dominance. (p873)

IV. Pharmaco*kinetics* and Pharmacogenetics

B. Metabolism – Description and 2 Phases

0. Overview

a. Lipophilic drugs metabolized → (relatively) hydrophilic metabolites (p867)

i. Lipophilic distribs more easily, hydrophilic excreted more easily

b. Prodrugs → metabolites (e.g. codeine, or toxic metabolites)

c. No relation b/n phase names (below) and order of occurrence

1. Phase I: Oxidation, Reduction, and Hydrolysis (p867)

a. Table of example enzymes and their representative drug metabolites (p868)

i. Cytochrome P-450 2D6 (aka CYP2D6) (e.g. codeine, debrisoquin)

ii. Cytochrome P-450 2C9 (CYP2C9) (e.g. warfarin)

iii. Cytochrome P-450 2C19 (CYP2C19) (e.g. omeprazole)

b. Cytochrome P-450 2D6 (CYP2D6) Pharmacogenetics

i. >75 known alleles, auto rec inheritance (p869)

c. 3 distinct groups of metabolic abilities due to allele differences or gene copy #s

*note: he uses 2 diff drugs to illustrate diff characteristics of the same enzyme*

i. CYP2D6 and Debisoquin (p869)

(a) Ultra-rapid, Extensive intermediate, Poor metabolism

(b) Crazy graph (p870); note x-axis is logarithmic, y-axis is # of people

ii. CYP2D6 and Nortriptyline

(a) More fxnal copies of the gene → higher rate of metabolism (p870)

(b) Ultra-rapid metabolizers can have sub-therapeutic responses to active drugs (inactivated too fast), but may have too high of a response to pro-drugs (activated all at once) (p871)

2. Phase II: Conjugation Reactions

a. Table (p872)

b. Thiopurine S-Methyltransferase (TPMT) (p872)

i. Mercaptopurine and azathioprine

(a) Prodrugs that → purine anti-metabolites

(b) Immunosuppressants (childhood acute lymphoblastic leukemia; ALL)

ii. TPMT metabolizes the active metabolites of these drugs → inactive forms

iii. Trimodal distribution: either TPMTH or TPMTL (p873)

(a) What happens to homozyg low-activity when given those drugs? What abt homozyg high-activity?

(b) TPMT\*3A (p874)

3. Therapeutic vs Toxic Doses (p876)

a. Pharmacogenetic diffs most pronounced when variation b/n therapeutic range is small

4. Poor Metabolizing Variant Genes assoc’d c Adverse Drug Reactions (table p877)

C. Drug Transporters

1. Example: P-Glycoprotein Transporter (p877)

o. Energy dependent cellular efflux of many substrates, including some drugs.

a. There are 2 SNPs in the gene for this (*ABCB1* aka *MDR1*) that exhibit strong linkage disequilibrium, so we don’t know which is the important one (p878)

b. One of them affects (i.e. lowers) plasma concentrations of Nelfinavir (antiretroviral), and leads to greater efficiency of the drug.

b. Key (I think) is that transporters can exhibit genetic differences too.

V. Pharmaco*dynamics* and Pharmacogenetics

A. Differences with Pharmacodynamics

1. “Dealing with variants that are NOT involved in determining the AMOUNT of a drug reaching the target(s)” (p878)

B. Genetic Variants in Drug Target Genes

1. Common drug targets: (p878)

a. Receptors (e.g. β-adrenergic, dopamine, epithelial growth factor)

b. Proteins c enzymatic activity (e.g. angiotensin converting enzyme; ACE)

C. Table of Genetic Variants in Disease or Treatment – Modifying Genes

D. β1-Adrenergic Receptor (ADRB1)

1. Drugs:

a. Agonists (Isoproterenol, dobutamine) (p880)

b. β-blockers = antagonists

2. Activation of ADRB1 (p880)

a. Stims rate/strength of heart contractions, lipolysis in adipocytes, release of renin in kidney

b. After stimulation, receptors “desensitize” and can be down-reg after prolonged exposure.

3. Variants

a. Arg-389 or Gly-389 (p881)

i. Bucindolol vs Carvedilol (β-blockers), which genotype responded better to which drug?

E. Epidermal Growth Factor Receptor (EGFR)

1. Gefitnib (Iressa) (tyrosine kinase inhibitor) improved non-small cell lung CA in small group of pts (p883)

a. Sequencing showed they all had activating mutations in EGFR gene

b. Some pts relapsed; developed 2° mut in EGFR activation gene, inactivating it (p884)

c. Gefitnib not effective over entire population, only in small portion c that mut.

VI. Genome-Wide Association Studies and Pharmacogenomics

A. Drug-Response-Affecting Genes Table (p885)

B. Adverse Drug Reactions Table (p886)

VII. Pharmacogenetics and Personalized Medicine

A. Abacavir and HLA Genotyping (p886)

1. FDA mandated genotyping

2. HLA-B\*5701 → life-threatening hypersensitivity rxn

a. Gene has lots of variation across diff populations (p887)

B. Clopidogrel (Plavix) and *CYP2C19* Genotyping (p888)

1. 25% of pts have subtherapeutic response (clopidogrel = prodrug) linked to *CYP2C19*

a. More Asians are poor metabolizers

2. Genotype of pt could → altered dosing schedule → improved treatment

C. Warfarin and Genotyping for *CYP2C9* and *VKORC1* (p889)

1. Both genes can affect warfarin response; genotyping → improved treatment (~30% fewer hospitalizations)

D. Table of Drugs that have included genetics in their prescribing information (p890)

VIII. Ecogenetics

A. Pts can respond differently to non-drug agents based on their genotype (p891)

B. Examples

1. Dietary

a. G6PD Deficiency (p891-2)

i. G6PD helps in pathway to convert G6P → glutathione, needed for reduction of oxidative stressors.

ii. Normally, only low lvls G6PD necessary, so pts c deficiency due to genetic diffs are generally phenotypically normal.

iii. However, environmental factors that can rapidly deplete G6PD lvls (e.g. oxidant drugs, and Fava beans) can result in problems for those pts

iv. Oxidative dmg in RBC (due to lack of glutathione) → jaundice, non-spherocytic hemolytic anemia

b. Dietary Lactose (p893)

i. Minichromosome maintenance 6 (*MCM6*) is very close to lactase gene (*LCT*), can affect lactose metabolism.

c. Dietary Folic Acid (p893)

i. Methylene tetrahydrofolate reductase (*MTHFR*)

2. Chemical and Physical

a. Inhaled Tobacco Smoke (p893)

i. α1-antitrypsin

b. Ultraviolet Light (p893)

i. Albinism or deficiencies in DNA repair enzymes